Spatial distribution and temporal progress of *Huanglongbing* in areas with strict disease management

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I dedicate with love to
my parents and sister Ivo, Ju e Bruna Pazolini,
my lovely grandparents Claudino e Onires
and my aunt Gemile
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“A viagem não acaba nunca. Só os viajantes acabam.

(...) O fim de uma viagem é apenas o começo de outra.

É preciso ver o que não foi visto, ver outra vez o que se viu já...”

José Saramago
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RESUMO

Distribuição espacial e progresso temporal de *Huanglongbing* em áreas com manejo rigoroso da doença

A citricultura é uma importante atividade agroindustrial para o Brasil e grande parte da produção se concentra no estado de São Paulo. Entretanto, a produção de laranja enfrenta, atualmente, uma epidemia de *Huanglongbing* (HLB), doença considerada a principal ameaça à citricultura mundial. Apesar de muitos esforços no manejo, a incidência de HLB continua aumentando desde a sua detecção no estado de São Paulo, em 2004. A dificuldade de manejo de HLB está associada à migração do inseto vetor, *Diaphorina citri*, que adquire a bactéria em fontes de inóculo externas e ao efeito limitado do manejo rigoroso da doença realizado pelas fazendas (erradicação de plantas sintomáticas e controle frequente do vetor) sobre a disseminação primária da doença. Assim, estudos epidemiológicos podem facilitar o entendimento do complexo patossistema e auxiliar na decisão de medidas de controle mais eficientes. Os objetivos desse trabalho foram: 1) determinar o padrão espacial de HLB em 24 blocos cítricos sob o manejo rigoroso da doença e 2) estudar o progresso temporal da doença e a distribuição espacial de HLB e do vetor em uma propriedade de larga escala que realizou o manejo rigoroso da doença e sua associação com fontes externas de inóculo. Para verificar o padrão espacial de HLB, utilizou-se a análise função-K de Ripley modificada e mapas de densidade de kernel. Os resultados demonstraram que o padrão de distribuição regular de plantas sintomáticas de HLB prevaleceu nos blocos estudados e uma agregação fraca de aproximadamente 58 m foi observada nas bordas dos blocos. As maiores distâncias de agregação e médias de *D. citri*/armadilha ocorreram em blocos localizados na periferia das propriedades. O padrão espacial regular e a concentração da doença e do vetor na periferia da propriedade evidenciaram a importância da disseminação primária na área. Para o estudo do progresso temporal, os modelos Gompertz e logístico foram ajustados ao progresso anual da doença de 177 blocos. A distribuição das taxas de progresso e incidência de HLB (para 177 blocos) e a média de *D. citri*/armadilha (para 296 blocos) foram visualizadas em mapas de gradiente. Os valores encontrados de taxa de progresso da doença, obtida com o modelo de Gompertz (de 0,04 a 0,28 por ano), incidência média de HLB (0,11) e média de *D. citri*/armadilha (0,016) foram baixos em comparação com dados da literatura. As variáveis apresentaram os maiores valores para blocos localizados na periferia da propriedade do que para blocos rodeados por citros. Uma grande quantidade de plantas não comerciais, e potencialmente servindo como fonte de inóculo, foi encontrada perto de blocos que apresentam as maiores taxas de progresso e incidências de HLB e médias de *D. citri*/armadilha. Os resultado encontrados demonstraram a importância da disseminação primária em áreas que realizam o manejo rigoroso de HLB. Portanto, para reduzir novas infecções, o manejo do HLB precisa reduzir efetivamente as fontes externas de inóculo e o manejo regional deve incluir, além de pomares comerciais, plantas não comerciais.

Palavras-chave: Efeito de borda; Manejo regional; Fontes externas de inóculo; Plantas não comerciais
ABSTRACT

Spatial distribution and temporal progress of *Huanglongbing* in areas with strict disease management

Citriculture is an important agroindustrial activity for Brazil and citrus production is concentrated in São Paulo State. However, the activity is facing a *Huanglongbing* (HLB) epidemic which is considered to be the main threat to global citrus production. In spite of many management efforts, the incidence of HLB continues to increase since its detection in São Paulo State, in 2004. The difficulty of handling HLB is associated with the migration of the vector, *Diaphorina citri*, which acquires the bacteria in external inoculum sources and the limited effect of the strict HLB management performed by farms (eradication of symptomatic trees and frequent vector control) on the primary dissemination of the disease. Thus, epidemiological studies can facilitate the understanding of the complex pathosystem and help in the decision of more efficient control measures. The objectives of this study were: 1) to determine the spatial pattern of HLB in 24 citrus blocks under strict management of the disease and 2) to study the temporal progress of the disease and the spatial distribution of HLB and the vector in a large scale property that carried out the strict management of the disease and its association with external inoculum sources. To verify the spatial pattern of HLB, modified Ripley’s K-function and kernel density estimation maps were used. The results showed that the regular distribution pattern of HLB symptomatic trees prevailed in the blocks studied and a weak aggregation of approximately 58 m was observed at the edges of the blocks. The largest aggregation distances and averages of *D. citri*/trap occurred in blocks located at the periphery of the properties. The regular spatial pattern and the concentration of disease and vector in the periphery of the properties evidenced the importance of primary dissemination. For the study of the disease temporal progress, the Gompertz and logistic models were adjusted to the annual progress of HLB for 177 blocks. The distribution of HLB progress rates and incidence (for 177 blocks) and average *D. citri*/trap (for 296 blocks) were visualized on gradient maps. The disease progress rate values obtained with the Gompertz model (0.04 to 0.28 per year), average HLB incidence (0.11) and average *D. citri*/trap (0.016) were low compared with literature data. The variables presented the highest values for blocks located at the periphery of the property, rather than for blocks surrounded by citrus. Many noncommercial plants, potentially serving as source of inoculum, were found near blocks with the highest rates of disease progress, HLB incidence and average *D. citri*/trap. The results demonstrated the importance of primary dissemination in areas that carry out a strict HLB management. Therefore, in order to reduce new infections, HLB management must effectively reduce external sources of inoculum and regional management should include, in addition to commercial orchards, noncommercial plants.

**Keywords:** Edge effect; Regional management; External inoculum sources; Noncommercial plants
1. INTRODUCTION

Huanglongbing (HLB) is the most devastating disease of citrus and has spread to the main growing areas worldwide, including Brazil, USA and China. HLB is associated with three species of phloem-restricted proteobacterias, ‘Candidatus Liberibacter africanus’, ‘Ca. L. asiaticus’ and ‘Ca. L. americanus’ (Gottwald, 2007). Two insect vectors are responsible for the fast spread of the disease: the Asian citrus psyllid (ACP), *Diaphorina citri*, in Asia and America, and the African citrus psyllid, *Trioza erytreae*, essentially in Africa (Duran-Vila & Bové, 2015). *D. citri* has been associated with citrus in Brazil since 1942 (Costa Lima, 1942), while the detection of ‘Ca. L. spp.’ occurred in 2004 in the central area of São Paulo State (Coletta Filho *et al*., 2004; Teixeira *et al*., 2005).

HLB epidemics has impacted citrus cultivation from individual farms to national economies, therefore impacting food security (Bergamin Filho *et al*., 2016) by declining fruits production and quality (Gottwald *et al*., 2010). Furthermore, the disease is responsible for shortening the life span of orchards (Das, 2004) and increasing productions cost, often leading to economic unfeasibility of citrus activity. In Brazil, it was estimated that more than 40 million HLB symptomatic trees had been removed by 2016 (CDA, 2017) and that more than 35 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt region, main orange juice producer region in Brazil (Fundecitrus, 2018).

There are no viable curative strategies or resistant citrus variety to control HLB yet, and disease management is based in the reduction of inoculum, i.e., utilization of healthy nursery trees, eradication of symptomatic trees, and chemical control of vector populations (Belasque *et al*., 2009). However, even in groves applying this management, HLB can cause infection in 100% of trees in 2 to 5 years (Bassanezi *et al*., 2013; Belasque *et al*., 2010). The low efficiency managing HLB has being related to the disease primary spread. While the secondary spread, i.e., the transmission of the pathogen to trees on the same orchard, can be reduced by insecticide sprays, the primary spread, i.e., the pathogen entry in property edges by the vector migration, is very difficult to control and, therefore, is responsible for introducing the disease even in areas that perform rigorous management. According to Gottwald *et al*. (2010), the primary spread is the most dangerous kind of spread as it indicates a spatial process of long distance, and then explain why control efforts on a local scale have always failed.

Taking into account the importance of HLB to national economy and the difficult in reducing disease incidences, epidemiology can be an important tool to gain valuable information and improve HLB management strategies. Detailed information on the spatio-temporal dynamics of plant diseases, i.e., estimation of the key epidemiological parameters, enables a more complete picture of
the structure and behavior of the pathosystem. This information can be used to predict the likelihood and extent of further spread, to describe and understand the development of diseases, developing sampling plans, planning controlled experiments, to characterize losses caused by the disease and to quantify the effectiveness of different strategies for disease control (Bergamin Filho et al., 2002; Parry et al., 2014).

Therefore, the objectives of these studies were to characterize the spatial and temporal progress of HLB in properties with a strict disease management in São Paulo State. These analyses in Brazilian conditions are scarce and can bring important information regard to HLB epidemic characterization. Furthermore, these findings can help to analyze current HLB control program as well to improve disease management strategies.
REFERENCES


2. LITERATURE REVIEW

2.1 Importance of HLB

*Huanglongbing* (HLB) is considered the most devastating disease of citrus and is present in many producing regions worldwide, including the three major citrus-producing countries: Brazil, the USA and China (Bové, 2014). The effects of HLB have impacted citrus production from individual farms to national economies by quickly declining fruits production and quality (Gottwald, 2010; Farnsworth *et al*., 2014), shortening the life span of orchards (Das, 2004) and increasing production costs. The citrus activity become unprofitable for many small and medium producers, leading to abandonment of orchards and causing a thousand of direct and indirect unemployment (Hodges & Spreen, 2012). Furthermore, there is no commercial variety of canopy or rootstock resistant to HLB and infected plants cannot be cured, yet. All these factors have made the citrus production a challenge.

According to da Graça *et al.* (2016) and Bergamin Filho *et al.* (2016), HLB is a complex disease that involves interactions among the pathogen, vector, hosts and the environment in its broadest definition. Moreover, the disease also account with a long incubation period, the inability, until now, to culture the bacteria, and the lack of any known sources of natural resistance (da Graça *et al*., 2016). These factors can explain why the disease has being causing so many losses since its detection, almost 100 year ago, and is still epidemic.

HLB was first reported in China, in 1919, but most likely originated in Taiwan in the 1870s (Bové, 2006; Lin, 1956). It was estimated that more than 100 million infected citrus trees have been destroyed by the disease throughout Asia (Zhang *et al*., 2014). USA and Brazil, two relatively new areas where the disease was detected, had the pathogen first report in 2004 in the central area of São Paulo State (Coletta Filho *et al*., 2004; Teixeira *et al*., 2005) and in 2005 in South Florida.

In Florida, 80% of orange trees were estimated to be HLB-infected (Singerman & Useche, 2016). Since the disease detection, HLB has reduced citrus area and yields by 26% and 42%, respectively, causing a devastating impact on industry (Institute of Food and Agricultural Sciences, 2016). According to Spreen & Baldwin (2013), HLB had caused an estimated loss of $1.3 billion in direct revenue and $3.6 billion in indirect revenue until 2013. In Brazil, it was estimated that more than 40 million trees had been removed by 2016 (CDA, 2017). It was also recently estimated that 35.3 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt region, main orange juice producer region in Brazil (Fundecitrus, 2018a).
Citrus Belt region is composed of São Paulo and west-southwest of Minas Gerais States. According to the inventory carried out by Fundecitrus in 2018 (Fundecitrus, 2018a), Citrus Belt region is composed by 5,882 orange growing properties, of which 64% are composed of less than 10,000 trees. Related to cultivated varieties, nearly 90% of the Citrus Belt is formed by five oranges: Pera Rio (35%), Valencia (27%), Hamlin (11%), Natal (11%) and Valencia Folha Murcha (4%). In the same region, the citrus planted area showed a decrease of 43,933 ha (7%) in 2018 comparing with 2015.

The reduction of citrus area in Citrus Belt is related, in part, to the abandonment of the activity mainly by small and medium producers. Low citrus prices, the high costs in the management of HLB and the fast reduction of productivity of infected orchards made the activity economically unfeasible and forces many producers to abandon the activity. According to the inventory, there are currently 6,050 ha of abandoned orchards in Citrus Belt. In Florida, this number was estimated to be 52,886 ha in 2016 (USDA, 2017). Cumming & George (2009) related the number of abandoned groves in Florida with socioeconomic pressures, freezing events, and the loss of production because of canker and HLB diseases.

Abandoned areas or areas with infected trees and no HLB management become inoculum sources and puts at risk the properties in which the strategies of HLB control are effectively employed (Bassanezi et al., 2013; Spreen & Baldwin, 2013). It is known that the vector D. citri is able to disperse several kilometers without wind assistance (Martini et al., 2013, 2014; Lewis-Rosenblum et al., 2015) and move frequently from abandoned to managed orchards (Boina et al., 2009; Tiwari et al., 2010; Lewis-Rosenblum et al., 2015). The movement of an infective D. citri from an orchard where it acquired the pathogen to another orchard is called primary spread.

Besides abandoned areas or areas with no HLB management, the maintenance of infected trees on sidewalks and backyards can be an even bigger problem to HLB management. These plants do not receive any chemical treatment for vector control and are often unnoticed as important sources of inoculum. Because of the importance of these trees, Fundecitrus, a citrus growers association, launched, in 2017, the “United Against Greening” campaign to raise awareness of the society on the impact of HLB and the need of inoculum sources removal (Fundecitrus, 2018b).

In the search for ways to reduce the devastating effects of HLB in citriculture, many changes are and still will be made so that the production of citrus keeps viable. According to da Graça et al. (2016), keep an economic production of citrus is the largest challenge ever faced by the citrus industry worldwide. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infections and the symptoms progress on citrus trees.
2.2 HLB etiology and symptomatology

HLB is associated with three species of phloem-restricted proteobacteria, ‘Candidatus Liberibacter africanus’ (Claf), ‘Ca. L. asiaticus’ (Clas) and ‘Ca. L. americanus’ (Clam) (Gottwald, 2007). In Brazil, two Liberibacter species are associated with the disease, Clas and Clam. Although genetically closely related, Clas tolerates higher temperatures, is more adapted to citrus and more efficiently transmitted by psyllid than Clam (Lopes et al., 2009a,b; da Graça et al., 2016). Since the detection of Clam in Brazil, in 2005, its occurrence decreased from 98 to 20% in a period of four years, and Clas is currently predominant (da Graça et al., 2016).

HLB bacterium have not been maintained in sustained pure culture until now (Davis et al., 2008) and its characterization was achieved around 1990s with DNA-based techniques (PCR and 16SrDNA). The pathogen was, then, confirmed to be Gram-negative bacteria belonging to a new genus ‘Candidatus Liberibacter’ in the alpha subdivision of the Proteobacteria (Bové, 2014).

The three species of ‘Candidatus Liberibacter’ are transmitted from plant to plant by grafting, psyllid vectors or experimentally by dodder. Two species of citrus psyllids are associated with HLB transmission: the Asian citrus psyllid (ACP), Diaphorina citri, in Asia and America, and the African citrus psyllid, Trioza erytreae, essentially in Africa (Duran-Vila & Bové, 2015). The ACP is a phloem specialist vector that acquires and transmits the bacteria while feeding on flush shoots of rutaceous host plants. Therefore, psyllids are essential to natural spread of the disease.

All known citrus varieties are susceptible to HLB (Folimonova et al., 2009). The ornamental rutaceous Murraya paniculata (orange jasmine) also has been confirmed as a host for both, Clam and Clas, in Brazil (Lopes et al., 2005, 2006). The bacteria were confirmed to multiply in psyllids and in the hosts (Lee et al., 2015).

After transmitted, the pathogens are carried through the flow of the sap to the entire plant, blocking the transport of sucrose to the root system and causing starch accumulation in the foliage, deterioration of the root system and nutrients deficiency symptoms (Kim et al., 2009; Etxeberria et al., 2009). The highest concentrations of Clas are found in the stem and midribs of flush (Lee et al., 2015). HLB symptoms can be seen in any period of the year but are more often between early spring and late summer. Initial HLB symptoms include yellow branches sparsely foliated with mottled leaves and small lopsided fruits. Infected branches produce small, misshapen and bitter fruits. According to Bassanezi et al. (2017), infected fruits have lower percentage of juice and juice can be 5 to 45% more acidic. HLB symptomatology evolves to severe defoliation, fruit drop and death of the branches (Bové, 2006; da Graça et al., 2016). Tree death may occur as result of defense gene expression in the plants, which cause the blockage of the phloem (Kim et al., 2009; Folimonova et al., 2009).
The latency period, i.e., the time between infection and the time that the pathogen is accessible to another vector acquisition, can be as short as 15 days (Lee et al., 2015). Unlike many other diseases, the latent and the incubation periods (onset of symptoms) can be vastly dissimilar (Boina & Bloomquist, 2015). While the latent period is as short as a single generation of psyllids (15 days), the incubation period can stretch from months to 6 years (Shen et al., 2013). The latent period depend on the age and nutritional status of the infected tree, bacterial and citrus species and climate conditions (Bové, 2006; Gottwald, 2010b; Shen et al., 2013). Consequently, trees infected at the same time may express the symptoms in different periods.

The incubation period of HLB is a limiting factor for the eradication practice. In addition to requiring trained staff for plant inspections, because the symptoms are very similar with symptoms of nutritional deficiency, inspections are useful only for symptomatic plants. Thus, while symptomatic trees are frequently removed in managed orchards, asymptomatic trees may be acting as inoculum sources.

### 2.3 Diaphorina citri

The vector Asian citrus psyllid (ACP) *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae) has been associated with citrus in Brazil since 1942 (Costa Lima, 1942), while both Clas and Clam were first described in 2004 in São Paulo State (Coletta Filho et al., 2004; Teixeira et al., 2005). Before detection of HLB, *D. citri* was considered a secondary pest. However, due to its ability to transmit HLB, the ACP is currently one of the most important vectors of world citriculture (Costa et al., 2010). In Florida, *D. citri* was first detected in 1998 (Halbert, 1998), but the psyllid quickly established in citrus producing regions and, by September 2000, had spread to 31 Florida counties (Halbert et al., 2001).

Development of ACP occurs in the presence of young citrus leaves where the female places its eggs and nymphs feed. Adults *D. citri* are able to feed on young and mature leaves, whereas early instars of *D. citri* feed only on flush shoots of rutaceous host plants (Hall & Albrigo, 2007; George et al., 2017). Thus, *D. citri* densities are strongly related with presence of new flush shoots (Yasuda et al., 2005; Tomaseto et al., 2015; Sétamou et al., 2016). The predilection of *D. citri* for young leaves is related, probably, to the higher concentrations of macro and micro nutrients and the ease of probing by the insect’s mouthparts (Sétamou et al., 2016; George et al., 2017).

Temperatures between 24°C and 30°C are the most favorable for insect survival and reproduction and, under this condition, adults life cycle lasts 30 to 50 days and females laid 500 to
800 eggs (Tsai & Liu, 2000; Nava et al., 2007; Hall et al., 2011). However, there are studies showing that *D. citri* can survive from 21 to 117 days (Liu & Tsai, 2000; Nava et al., 2007).

Both *D. citri* nymphs and adults can acquire the HLB pathogen when feeding on infected plants (Capoor et al., 1974; Xu et al., 1988), but it’s a poor vector when the pathogen is acquired during the adult stage, unlike adults emerged from nymphs that acquired the pathogen (Inoue et al., 2009; Pelz-Stelinski et al., 2010). It takes 5 to 7 hours of feeding to *D. citri* acquire the pathogen (Halbert & Manjunath, 2004) and only 40% of adults insects successfully acquire the bacteria (Pelz-Stelinski et al., 2010). Also, a latent period of 1 to 25 days may be required before transmission of HLB when it is acquired by *D. citri* adults (Huang et al., 1984; Pelz-Stelinski et al., 2010; Grafton-Cardwell et al., 2013). On the other hand, when acquired at the nymphal stage, adults are able to transmit HLB immediately after emergence (Inoue et al., 2009; Capoor et al., 1974; Xu et al., 1988). Clas is transmitted by *D. citri* in a persistent and propagative manner. According to Ammar et al. (2016), Clas appears to multiply in both, nymphs and adults, but adults may require longer time feeding on infected plants for Clas to reach higher levels in the vector.

Although nymphs are more efficient in acquiring and transmitting HLB, the adults play a more important role as they are responsible for disease spread. Its known that *D. citri* is able to disperse many kilometers without wind assistance (Martini et al., 2013, 2014; Lewis-Rosenblum et al., 2015) and, therefore, may disseminate HLB within and between groves (Halbert & Manjunath, 2004). According to Kobori et al. (2011), adults initiate dispersion 3 to 4 days after emerging.

Boina et al. (2009) verified that *D. citri* might move at least 100 m between groves in 3 days period and Lewis-Rosenblum et al. (2015) verified that the psyllids were able to disperse at least 2 km within 12 days. Also, geographical barriers are known to have little impact on the vector dispersal as the psyllid has been captured in a dense forest 2.3 km away from a citrus grove (Martini et al., 2013) and in urban environments (Chong et al., 2010; Godfrey et al., 2013).

Therefore, due *D. citri* abilities to disperse to long distances, abandoned citrus areas or unmanaged hosts play an important role as a reservoir for HLB (Boina et al., 2009; Tiwari et al., 2010; Lewis-Rosenblum et al., 2015). Furthermore, as studied by Tiwari et al. (2010), psyllids tended to migrate from abandoned plantings into managed groves. For these reasons, vector control and trees rouging must be performed in a regional scale (Bassanezi et al., 2013).
2.4 Management of HLB in Brazil

As already mentioned, there are no citrus commercial varieties of canopy or rootstock resistant to HLB and infected plants cannot be cured yet. According to da Graça et al. (2016), the lack of HLB natural resistance in citrus is one of the major factors contributing to the rapid spread and devastation of the disease. For now, integrated management is the most efficient way to reduce HLB effects and must be done simultaneously by all citrus growers in a regional scale to reduce primary spread effects (Bassanezi et al., 2013; Bergamin Filho et al., 2016). Since 2006, and based on other countries experience, three basic HLB management strategies have been indicated and adopted, at different levels, in São Paulo citrus properties: use of healthy nursery trees, inspections and eradication of symptomatic trees and chemical control of the vector, D. citri.

Contaminated citrus seedlings can be an important mean of spreading HLB, as the commercialization can occur at long distances and may introduce HLB into areas still free of the disease. Also, sooner the trees get infected, shorter will be the productive life of the orchard. Therefore, it is essential to acquire healthy nursery trees for formation of new orchards or to replanting eradicated trees.

The use of certified citrus seedling in São Paulo State was implemented after a Citrus Variegated Chlorosis (CVC, Xylella fastidiosa) epidemic, in 1990. Transmission of CVC by spittlebugs led the Department of Agriculture and Food Supply of the State of São Paulo to create a public normative, from 2000, regulating citrus propagating material production. The normative induced significant changes such as mandatory production of seedlings in insect-proof green houses and high standards of sanitation (CDA, 2017). With the implementation of these phytosanitary norms, the risk of introducing diseases in citrus orchards through seedlings was significantly reduced. When HLB was first reported (2004), São Paulo industry was already producing and planting certified nurseries seedlings. Thus, the HLB spread in Brazil is predominantly associated with the movement of D. citri.

Florida initiated the implementation of phytosanitary norms to production of seedlings in an ACP-free environment only in 2008 (Halbert et al., 2012), after HLB introduction, in 2005. The delay in seedlings certification is one of the main causes of the faster spread of the disease in the USA. While in Brazil it was estimated that HLB expanded, approximately, 20 km per year (Gottwald et al., 2007), in Florida, the estimation was 50-60 km per year (Halbert et al., 2008).

Regard to the eradication of symptomatic trees, frequent inspection of all trees is an important strategy aiming the early detection of HLB. Surveys should be performed by well-trained staffs, as disease initial symptoms can be easily confused with symptoms of nutritional deficiency. In orchards with adult trees, platforms of four inspectors (two observing the top and two observing the
skirt of the trees) are indicated (Belasque et al., 2009). However, even in such conditions, according to Bové (2014), only 60% to 70% of symptomatic trees are detected. Besides that, inspections of all trees by trained staffs are expensive and take a long time.

Even if a 100% of symptomatic trees were detected by visual inspections, and eradicated, it wouldn’t mean the total elimination of infected trees (inoculum sources), as HLB incubation period is variable. In this sense, techniques for identification of asymptomatic trees would be of great help so HLB infected trees could be eradicated as soon as possible. An early and fast diagnosis of ‘Ca. Liberibacter’ is critical in reducing the spread and devastation of HLB, as well as minimizing the economic impact of potential false-positive diagnoses (Valdés et al., 2016). Many efforts have been made for development of a fast and viable technique for HLB detection, such as real-time PCR (Orce et al., 2015), visible spectrum image (Deng et al., 2016), optical sensor (Mishra et al., 2011), among others.

Orange jasmine (Murraya paniculata; Rutaceae), an ornamental plant frequently used in Brazil and alternative host to D. citri and HLB bacteria, also need to be a target on a program of eradication and ACP control. This plant is an inoculum source in HLB epidemics and its importance shouldn’t be underestimated.

Among the three basic strategies for HLB management, eradication of symptomatic trees is the most controversial and difficult to accomplish by citrus growers (Martini et al., 2015). This activity is expensive as involves frequent symptom inspections, eradication of the symptomatic trees, direct impact on production by eradication of productive trees and costs of seedlings replanting (Parnell et al., 2010; Bassanezi et al., 2013b).

Different from trees eradication, the chemical control of D. citri is better accepted and carried out by most of the producers. The effective monitoring and control of the psyllid are essential for HLB management. The most frequent used method of monitoring ACP in Brazil are the yellow sticky cards (traps), but visual inspection of young citrus shoots is also used to detect the presence of eggs, nymphs and adults (Belasque et al., 2010). The greater detection of D. citri nymphs in branches may indicate a bad chemical management, as the developmental period from egg to adult may be as short as 14 days (Liu & Tsai, 2000).

Monitoring the vector population is an important activity for the decision of insecticide applications (Bassanezi et al., 2013), and so, at least one trap per citrus block or a regular distribution of traps is indicated in the property. Also, periods of the highest vegetative activity of the crop, when control measures may be more efficient due to the lower insect activity, must be taken into account (Tomaseto et al., 2015). Flushes at emerging and developmental phases offer optimal conditions for feeding and oviposition of D. citri and are attractive by a combination of chemical volatiles and visual stimuli (Patt et al., 2014; Patt & Sétamou, 2010; Wenninger et al., 2009).
Usually, *D. citri* detection (Sétamou & Bartels, 2015) and HLB incidences (Bassanezi *et al.*, 2013; Bergamin Filho *et al.*, 2016; Gasparoto *et al.*, 2018; Monteiro *et al.*, 2013) are concentrated on the border of the properties (border effect). Thus, many citrus growers intensify the management in the border of the citrus blocks, by increasing the frequency of insecticide applications in these areas (Miranda *et al.*, 2018). Besides contact insecticide sprays, systemic insecticide treatment of young orchards, until three year old, is highly indicated due the higher vegetative activity in the trees. (Bové, 2014; Rogers & Shawer, 2007; Sétamou *et al.*, 2010).

However, even a property carrying a very strict management of *D. citri*, with use of healthy seedling, frequent inspections and HLB-tree eradication and frequent insecticide sprays, it won't achieve total prevention of primary infections due insect's ability to dispersal among citrus groves (Halbert & Manjunath, 2004; Boina *et al.*, 2009; Martini *et al.*, 2013, 2014; Lewis-Rosenblum *et al.*, 2015). The high rates of HLB spread and external inoculum sources are the main difficulties of controlling the disease (Bové, 2014; Gottwald *et al.*, 2010b).

Therefore, for successful HLB management, the removal of symptomatic trees and psyllid control must be accomplished regionally by citrus growers, and include residential citrus and abandoned orchards (Bassanezi *et al.*, 2013; Bergamin-Filho *et al.*, 2016). However, an area-wide management is not simple to accomplish due the difficulty for coordinating control actions among neighboring farmers and government regulation is required (Bergamin Filho *et al.*, 2016; Tomaseto *et al.*, 2017). Seeking to facilitate an area-wide coordinate management, programs developed by Fundecitrus, such as the “Phytosanitary Warning System” and the "United Against Greening" campaign have been assisting citrus growers.

The “Phytosanitary Warning System” organizes information of the regional monitoring of *D. citri* populations by georeferenced traps and vegetative stage of citrus plants. The program alerts producers of critical moments for regional control of *D. citri*. The "United Against Greening" campaign aims to raise awareness of the society on the impact of HLB and the need of removing hosts plants on sidewalks or backyards (Fundecitrus, 2018b). These plants do not receive any chemical treatment for ACP control and are often unnoticed as important sources of inoculum.

An important alternative control method of *D. citri* that had been widely used in the regional management is the ectoparasitoid biocontrol agent *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae). A single ectoparasitoid is able to parasites up to 500 nymphs (Skelley & Hoy, 2004) with a parasitism rate of more than 77% (Gómez-Torres *et al.*, 2012). The parasite is very useful on regional management as the insects can be released on areas with inoculum sources that are unknown, such as woods, or areas where eradication and sprays with insecticides are not allowed, such as backyards.
Some other important aspects to the management of HLB are the size and shape of the properties, age of the orchard, incidence of HLB in the area where the property is located and presence of neighboring properties with no HLB management. Due the border effect, small or narrow orchards will be more affected due the greater periphery area than large and square farms (Bové, 2014). Many times, citrus production may lose economic viability to small producers, what can lead to a dangerous cycle: abandoned properties became HLB inoculum sources to others properties (Bové, 2014).

With the lack of resistant cultivars and curative methods, HLB epidemics can be relatively fast and destructive for local citrus industries if no effective control measures are undertaken immediately (Bassanezi & Gottwald, 2009). However, to keep citrus orchards producing without contracting or with low HLB incidence is costly and may be difficult even under intensive management programs. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infection and the symptoms progress on citrus trees.

2.5 Epidemiology of HLB

Although the high and increasing HLB incidences and the concerning situation of the agribusiness chain of citrus, relatively few quantitative epidemiological studies have been conducted in Brazil. Epidemiology is an important tool to predict the likelihood and extent of further spread, to describe and understand the development of diseases, developing sampling plans, planning controlled experiments, to characterize losses caused by the disease and to quantify the effectiveness of different strategies for disease control (Bergamin Filho et al., 2002; Gottwald et al., 2010a; Parry et al., 2014).

However, studying HLB epidemiology can be a difficult task due, mainly, the variable lag on the HLB expression of the symptoms, the perennial nature of the disease and host and the difficulty to locating study sites where the disease is allowed to progress without intervention of control activities (Bassanezi et al., 2010; Gottwald et al., 2010a). Although accuracy of temporal and spatial studies can be affected by these factors, useful information can still be obtained (Gottwald et al., 2007; Gottwald, 2010b).

Temporal dynamics of HLB were investigated, initially, in Reunion Island and Southern China (Gottwald, 1989; Gottwald et al., 2007). Gottwald et al. (2010b), using logistic and Gompertz linear models to explain HLB epidemic in Florida, obtained rates varying from 0.72 to 2.73 for the
logistic model and 0.43 to 0.97 for Gompertz model. Bassanezi et al. (2013a) and Bassanezi et al. (2013b), obtained HLB rates ranging from 0.82 to 2.77 through linear regression of the logistic model in citrus blocks under strict management in Brazil. Arruda (2017) found logistic rates ranging from 0.28 to 1.88 and Gompertz rates ranging from 0.13 to 1.00, using non-linear regression, on data of HLB epidemic progress in properties under strict HLB management in São Paulo. According to Bassanezi and Gottwald (2009), taking into account the perennial nature of citrus plantings, HLB epidemics can be considered a fast disease. In absence of management in young plantings, disease incidence can reach more than 50% of the trees in 3 to 5 years.

Analysis of spatial distribution of infected trees had also brought important information in the understanding of the HLB pathosystem. According to Taylor (1984), the knowledge of the spatial distribution of a disease provides valuable references to the main factors that explain its nature. These analyses are essential for understanding the behavior of an infection between sick plants at near and long distances, as well as the influence of the vector in both processes (Gottwald et al., 2007). According to Shen et al. (2013), understanding spatial patterns of HLB spread may help identify control measures to reduce the spread of disease and address questions on where the greatest risk is and how it is likely to be reduced in certain locations.

A characteristic of the spatial distribution of HLB is the edge effect, i.e., disease incidences are characteristically higher on the border of properties. The edge effect is caused by the migration of infective psyllids from nearby areas without or poor HLB management and, as a consequence of D. citri migration, most of the symptomatic trees can be seen on the peripheral areas of the properties. Therefore, the border effect is an indication of psyllid migration (primary spread). This characteristic in the spatial distribution of plants infected with HLB has already been observed and analyzed by several researchers (Bassanezi et al., 2005, 2006; Gottwald et al., 2008; Gottwald, 2010b; Gottwald et al., 2007; Sétamou, 2014; Sétamou & Bartels, 2015; Shen et al., 2013; Tomaseto et al., 2015). Gottwald et al. (2008) also point out that besides the edges, infections caused by HLB tend to occur in areas of orchards intersected by roads, canals, containers, houses, buildings and equipment.

Gottwald et al. (2010a), using spatio-temporal Markov-Chain Monte Carlo (MCMC) analysis to study the spread of HLB in citrus blocks in south Florida, verified that spatial progress of HLB can occur due to only primary or secondary dissemination, but, mostly, it occurs as an incessant mixture of both spatial processes. Despite both processes importance to HLB epidemic, secondary spread is easily reduced by insecticide sprays, but the pathogen entry in property edges, carried by vectors, is very difficult to control. Thus, even properties performing intensive HLB management are constantly re-infested and subject to continuous primary infections, especially in the borders of the groves (Bergamin Filho et al., 2016), and a regional management is necessary (Bassanezi et al., 2013b; Bergamin Filho et al., 2016; Gasparoto et al., 2018).
The presence of properties without or with poor HLB management have been a major problem for efficient HLB management (Bové, 2014). According to Gottwald et al. (2010a), the primary dissemination is the most dangerous kind of spread because it indicates a spatial process of long distance or regional spread, and then, it explains why control efforts on a local scale have always failed. This was later confirmed by Bassanezi et al. (2013), which demonstrated that the control effects of the primary dissemination is only possible with an area-wide inoculum reduction and vector control.

The spatial dynamics of HLB was investigated in many places, as the Reunion Island, China, Philippines (Gottwald et al., 1989; Gottwald et al., 1991; Gottwald et al., 1991), Brazil (Bassanezi et al., 2005, 2006; Leal et al., 2010), Cuba (Batista et al., 2013), the USA (Gottwald et al., 2008; Gottwald et al., 2008b,a; Gottwald, 2010; Shen et al., 2013b; Sétamou et al., 2014) and Mexico (Pèrez et al. 2016). In all studies areas, it was observed aggregation of the HLB diseased plants, except when the incidence was extremely low or high. In a study performed in Brazil, aggregation within quadrats was not observed in the majority of blocks when disease incidence was low, indicating that infective vectors land at random in a field at the beginning of the epidemic, before they become established in a tree (Bassanezi et al., 2005).

Evidences of HLB transmission among adjacent trees were demonstrated by the ordinary runs analysis. However, the percentage of aggregation within and across rows was low, being the within row aggregation slightly stronger than across-row aggregation (Gottwald et al., 1991; Bassanezi et al., 2005; Shen et al., 2013b). Therefore, vector movement appears to occur both from one tree to those within the immediate vicinity, and over a larger scale to trees at 25 to 50 m distance, the latter initiating new foci of infection (Gottwald et al., 1989; Gottwald et al., 1991; Bassanezi et al., 2005).

Gottwald et al. (2010b) used modified Ripley’s K-function method to present the first regional examination of spatial distribution of HLB. Data was obtained from a 4,800 ha plantation in South Florida where infection was entirely dependent on psyllid transmission. Modified Ripley’s K-function analyses demonstrated a continuous relationship between HLB diseased trees in a range up to 3.5 km. A range from 0.88 to 1.61 km was also indicated as the most common distance between pairs of diseased trees, which may indicate a mean distance of psyllid regional dispersion. Modified Ripley’s K-function describes the interaction or spatial dependence between points through space (Ripley, 1981). The analysis pairwise distances between diseased trees to assess clustering versus regularity and has been widely used in the study of plant diseases, including citrus canker (Gottwald et al., 2002) and citrus black spot (Spósito et al., 2007; Hendricks et al., 2017).
Epidemiological analyses of HLB in Brazil, such as modified Ripley’s K-function, are scarce. However, this information is important for understanding the epidemic in Brazilian conditions and to improve the disease control.
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3. SPATIAL PATTERN OF **HUANGLONGBING** EPIDEMIC UNDER STRICT MANAGEMENT IN SÃO PAULO, BRAZIL

*Huanglongbing* (HLB) is a complex citrus disease with a difficult and expensive management that have been concerning the agribusiness chain of citrus in many countries. Quantifying the spatial nature of the disease spread can be a key to understanding the epidemiology of the pathogen and improve control measurements. This study aimed to determine the spatial pattern of HLB in citrus blocks under strict disease management (trees eradication and vector control), belonging to three properties, in São Paulo State, Brazil. For this, modified Ripley’s K-function and kernel density estimation maps (KDE) were performed in 24 commercial citrus blocks (totaling 537.4 ha) where the spatial distribution of infection was assessed 3 to 5 times each, between 2013 and 2017. Cumulative HLB incidence on the 24 blocks ranged from 0.7 to 23.4%. Results of the modified Ripley’s K analysis demonstrated a regular distribution pattern of HLB infected trees prevailing in the range of distances studied (up to 434.0 m). Weak aggregation, with an average range of spatial dependency (RSD) between infected trees of 58.0 m, was observed on the 24 citrus blocks. The MAX distance, i.e., the most common distance between pairs of HLB infected trees was 38.1 m for blocks located internally and 51.0 m at the periphery of the properties. Higher averages of RSD and *D. citri* adults per trap were found in blocks located in the periphery of the properties. The KDE found higher density of HLB infected trees at the border of the citrus blocks, mainly at the periphery of the properties. These results demonstrated that, under strict management, HLB primary spread from surrounding areas was predominating on the 24 citrus blocks. Therefore, to reduce new infections, HLB management needs to effective reduce external inoculum sources and/or include strategies that reduce the entry of *D. citri* in the area.

**Keywords:** Ripley’s K-function; Edge effect; Regional management

3.1 Introduction

*Huanglongbing* (HLB) is considered the most devastating disease of citrus and is present in most of the producing regions worldwide, including the three major citrus-producing countries: Brazil, the USA and China (Bové, 2014). Where it occurs, HLB has been responsible for drastic reduction of citrus production and quality, citrus blocks abandonment, shortening the life span of citrus orchards and for increasing production costs (Gottwald *et al.*, 2010; Farnsworth *et al.*, 2014; Das, 2004). In Brazil, it was estimated that more than 40 million trees had been removed by 2016 (CDA, 2017) and 35.3 million orange trees (18.15%) are currently HLB symptomatic in the Citrus Belt area, main orange juice producer region in Brazil (Fundecitrus, 2018a).
There is no commercial variety resistant to HLB or viable curative control methods yet, and the integrated management is the most efficient way to reduce HLB effects. Three basic strategies has been indicated and performed in Brazilian properties, at different levels, since 2004 (Bové, 2006): planting of healthy nursery trees, inspections and eradication of symptomatic trees and frequent chemical control of the vector, the Asian citrus psyllid (ACP), *Diaphorina citri*. Among the strategies to control *D. citri*, insecticides sprays are the most effective and frequently used (Boina & Bloomquist, 2015).

However, even applying the indicated management, HLB can infect 100% of trees in 3 to 5 years (Bassanezi et al., 2013; Belasque et al., 2010). The indicated management can be very efficient against acquisition and transmission of the pathogen by the vector inside the property (secondary spread) when applied locally. On the other hand, these strategies can have little or no effect on the entrance on ACP that acquired the pathogen at inoculum sources around the property (primary spread) (Gatineau et al., 2010; Bassanezi et al., 2013a,b). The relevance of primary spread on HLB epidemiology is related to the ACP constant movement among citrus groves over short (<200m) and long (>2 km) distances (Boina et al., 2009; Lewis-Rosenblum et al., 2015; Martini et al., 2013, 2014). Thus, even properties performing intensive HLB management are constantly re-infested and subject to continuous primary infections, especially in the borders of the groves (Bergamin Filho et al., 2016).

The border effect is the result of the migration of infective psyllids from outside the grove and can represent from a small portion to a total area of a property, depending on groves shape and size. To reduce the higher incidences of HLB near the periphery areas of the properties, the area-wide management is necessary (Bassanezi et al., 2013b; Bergamin Filho et al., 2016; Gasparoto et al., 2018). Also, many citrus growers have been intensifying the chemical management in the border of the groves by increasing the frequency of insecticide applications in an attempt to reduce HLB incidences in these areas (Miranda et al., 2017). However, the efficiency of these applications is questionable since it effect on the reduction of HLB infected trees on the borders is unknown.

HLB is a complex disease and so, requires complex management measures. Epidemiological analyses are important tools that can bring valuable information on the understanding of complex pathosystems, such as HLB. Quantifying the spatial nature of spread of the associate bacterium can be a key to understanding the epidemiology of the disease, which can then be used to improve its control. Ripley’s K-function analysis has been widely used in the study of disease point pattern (Ripley, 1981), including HLB epidemic in commercial citrus blocks in Florida (Gottwald et al., 2010). By calculating the average density of healthy and diseased trees on a sequence of circles with increasing radius surrounding each tree, Ripley’s K-function detect the spatial pattern of the disease.

Despite the complexity in the management, the increasing disease incidences and the concerning situation of the agribusiness chain of citrus, relatively few spatial epidemiology studies...
have been conducted in HLB pathosystem. In this study, we analyzed the spatial pattern of HLB epidemic in 24 commercial citrus blocks, totaling an area of 537.4 ha, that were under strict disease management, in São Paulo State. The objective of this study was to determine the spatial pattern of HLB in such conditions and gain some information on disease spread, so better management strategies can be applied.

3.2 Material and Methods

3.2.1 Area and data

Locations of trees eradicated due HLB symptoms was assessed in 24 citrus blocks belonging to three commercial citrus properties (E, A and D) located in an HLB endemic region in São Paulo – Brazil (Figure 1). Data consists of more than 330,000 trees distributed among 24 rectangular citrus blocks, totaling 537.4 ha. All citrus blocks were separated by roads and each one was composed of ~60 rows and ~250 trees/row of sweet orange 'Pera Rio' (Citrus sinesis (L.) Osbeck). Citrus blocks characteristics are given in Table 1.
Figure 1. Maps of properties E, A and D and location of studied citrus blocks. Red dashed line represents properties periphery. Lilac, purple and green areas represent facilities, landing strips and citrus neighbors, respectively.
Table 1. Characteristics of 'Pera Rio' sweet orange citrus blocks studied in São Paulo, for HLB eradicated trees spatial pattern.

<table>
<thead>
<tr>
<th>Property</th>
<th>Block</th>
<th>Size</th>
<th>Rootstock</th>
<th>Spacing between row</th>
<th>Spacing between trees</th>
<th>Date of planting</th>
<th>Total trees</th>
</tr>
</thead>
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<td>23.87</td>
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Three to five successive evaluations (snapshots) were made, for each block, between 2013 and 2017. Snapshots were based in aerial photographs used to track the disease status of every three of the 24 citrus blocks. Excel binary matrices based on disease status of every tree of each area were prepared with 0 for preset (not symptomatic trees) and 1 for HLB eradicated trees. Each area contains 3 (blocks 37, 38, 39, 40, 51502, 51503, 51504, 51505, 51506, 51507, 51508), 4 (blocks 51101, 51102, 51501, 51403, 197, 198, 199, 200, 204, 205, 206) or 5 (blocks 33 and 34) snapshots, totaling 87 Excel matrices for the 24 blocks. Snapshots were cumulative and therefore were consisted of the new HLB eradicates trees as well as all previous eradicated trees from previous snapshots.
3.2.2 HLB management performed by the properties

The three properties were considered to perform a strict HLB management. For this, inspections and eradication of symptomatic trees and monitoring and chemical control of *D. citri* were performed frequently in properties E, A and D. Replacement of HLB eradicated trees were performed with healthy nursery trees produced in insect-proof nursery. The use of healthy nursery trees in the implementation of the orchards was accomplished by properties E and A. For property D, as reported by property managers, healthy nursery trees were exposed, before being planted and receive chemical management, near to highly infected citrus blocks, for a few days.

At least five inspections per year (average) were performed, by professional scouts, for HLB symptoms detection in canopies of each tree of each block (Table 2). PCR analysis was used for confirmation of infection when the symptomatology was not conclusive. Symptomatic trees were eradicated within 30 days after detection.

Table 2. Inspections per year for HLB symptomatic trees in the 24 citrus blocks.

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* Number of inspections in citrus blocks until Jul 2016
D. citri was monitored by observation of eggs, nymphs and adults in branches, by professional scouts, twice a monthly, on 1% of trees, three branches per tree. Also, each block of the properties contained, at least, one yellow adhesive trap (30 x 10 cm) fixed in the upper third of trees located at the border of the block for capture of adult psyllids. Traps were changed and analyzed weekly, by professional scouts. Analyzes of the traps were carried out directly in the field, until Sep 2014, and, after, inside facilities. Data of D. citri detection in traps, from Jun 2012 until Oct 2016, was provided by the three properties for this study.

D. citri control was performed with contact insecticides applied via terrestrial, aerial and at the borders of the citrus blocks. Terrestrial insecticide sprays were performed with turbo sprayers, at least twice a month (Table 3), following a pre-established spray program and were intensified according to increasing detection of D. Citri or Fundecitrus phytosanitary alert recommendations (Fundecitrus, 2018b). Aerial sprays (5.0 L/ha) were performed with airplanes Ipanema (application range of 18 m) or airplane Air Tractor (application range of 28 m), in the three properties (Table 4). Border insecticide sprays (Table 4) were applied, in all blocks of each property, with thermal foggers oriented from the outside into the blocks. In addition to contact insecticides, systemic insecticides were applied, via soil drench (200 to 1000 ml/tree), in young trees, until 3 years old (Table 5).
Table 3. Terrestrial insecticides sprays performed in the 24 citrus blocks.

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* Number of terrestrial sprays until Jul 2016
- Year when citrus block wasn’t planted yet

Table 4. Number of aerial and border insecticide sprays in properties PE, PA and PD.

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* Number of aerial and border sprays until Jul 2016
Table 5. Number of insecticide application via drench in the 24 citrus blocks.

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* Number of applications until Jul 2016
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3.2.3 Data analysis

Spatial pattern of HLB eradicated trees, of the 87 snapshots belonging to the 24 citrus blocks, was examined via modified Ripley’s K-function and kernel density estimation (KDE). For this, the 87 binary matrices based on disease status of the trees were converted in geographical coordinates information. Besides studying HLB spatial pattern in the 24 blocks individually, blocks 37, 38, 39 and 40 were studied grouped as a single area of 98.3 ha, called E2.

Modified Ripley’s K-function method was performed as described by Gottwald et al. (2002) and Gottwald et al. (2010) to indicate the spatial dependence between infected trees through space, i.e., aggregation or regularity. The analysis compared two cumulative distribution functions, one representing the infected trees spatial point pattern (cdfi) and the other, the distribution of the total
population of trees (cdfs). Both cumulative distribution functions were plotted against the distance of the radius analyzed. The significant difference between cdfs indicated the range of spatial dependency (RSD), i.e., the range of distance over which there was aggregation of eradicated trees. The analysis considered, for radio distance, the maximum distance to cover the shortest dimension of the block and varied from 77.5 to 434.0 m between the 24 blocks. The RSD and the distance at which maximal spatial difference (Max) occurred were calculated for the 87 snapshots belonging to the 24 citrus blocks, and for E2 area. Kernel density estimation (KDE) maps, applied for each of the 24 citrus blocks, were used to demonstrate where the HLB eradicated trees were concentrated.

Average RSD, HLB cumulative incidences and D. citri/trap of the 24 citrus blocks were statically compared by Kruskal-Wallis h test (p≤0.05) between the properties E, A and D and between the blocks located internally or at the periphery of the properties. Blocks 33, 37, 39, 40, 197, 198, 205, 501, 503, 505, 507 were grouped as internal, and blocks 38, 199, 200, 204, 206, 34, 101, 102, 403, 502, 504, 506, 508 were grouped as peripheral. Internal blocks were located, at least, 200 m away of the periphery of the properties, while peripheral blocks were facing external areas of the properties. Average D. citri/trap of all citrus blocks of each property (data of the entire properties) were also compared between properties E, A and D, which were composed of 314, 34 and 35 citrus blocks, respectively.

### 3.3 Results

Modified Ripley’s K-function analysis allowed accessing the range of spatial dependency (RSD) and the distance of maximum departure from randomness (MAX) for successive snapshots of HLB infection spatial distribution in a total area of 537.4 ha, that was under strict disease management. By applying this method to the 3 to 5 successive snapshots of the 24 citrus blocks and E2 area, it was observed a spatial pattern structure of HLB infected weakly related in very short distances and regular distribution pattern prevailed in the range of distances studied (77.5 to 434.0 m) (Figure 2). For the 87 snapshots analyzed, it was observed an average RSD of 58.0 m, ranging from 16.2 (block 200) to 221.0 m (block 204). The average MAX, i.e., the most common distance between pairs of HLB infected trees, was of 45.1 m, ranging from 11.6 (block 198) to 215.1 m (block 204).
Figure 2. Modified Ripley’s K-function analysis of HLB infection spatial pattern in commercial citrus blocks under strict disease management, in São Paulo State. Results of the last snapshot (cumulative incidence) of the blocks 51504, 40, 198, 204, 51403, and area E2 are presented. Infected trees spatial point patter (cdfi) and complete spatial randomness (cdft) are represented by the black and grey curves, respectively. Range of spatial dependency (RSD) and the distance of maximum departure from randomness (MAX) are represented by the solid black and dashed grey lines, respectively.
RSD distances between HLB infected trees, in the cumulative incidences, for all the 24 citrus blocks, are presented in Figure 3. The higher spatial dependency distances were observed in citrus blocks 38 (102.1 m), 204 (221 m), 205 (168.9 m) and 206 (87.6 m), all belonging to property E. Even so, the distances found can be considered very low. Properties E, A and D didn’t differ ($p>0.05$) in average RSD, which were 75.4, 47.5 and 40.1 m, respectively. On the other hand, the 24 blocks differed ($p\leq0.05$) when RSD was compared between internal (46.1 m) and peripheral (68.1 m) blocks. The MAX was 38.1 m and 51.0 m for blocks located internally and at the periphery of the properties, respectively.

![Maps of properties E, A and D with studied citrus blocks. Range of spatial dependency (RSD), HLB incidences and average D. citri/trap are presented in green, blue and orange, respectively. Data of D. citri detection from Jul 2012 until Oct 2016. Redline represents the property’s periphery. Lilac, purple and green areas represent facilities, landing strips and citrus neighbors, respectively.](image)

HLB cumulative incidences ranged from 0.7 to 23.4% of eradicated trees on the 24 citrus blocks (Figure 3). The higher average of HLB incidence was observed in the property D (14.0%) ($p\leq0.05$) when compared with properties E and A (2.4 and 4.5%, respectively). However, the average HLB incidences didn’t differ ($p>0.05$) between the blocks located internally (7.2%) or at the periphery of the properties (8.0%).
The average *D. citri*/*trap* was studied in two different scales: in the 24 studied citrus blocks and at the entire properties E, A and D. The average *D. citri*/*trap* in the 24 blocks varied between 0.000 and 0.146 (Figure 3). The blocks 33, 39, 40, 51403 and 51503 didn’t have *D. citri* detection in the period studied. No significant differences (*p*>0.05) were observed between average of *D. citri*/*trap* of the 24 blocks separated by properties E, A and D, which presented 0.03, 0.01 and 0.01 adults per trap, respectively. When separated by internals or peripherals, the 24 blocks differed (*p*≤0.05), presenting 0.01 and 0.03 *D. citri*/*trap*, respectively. The average *D. citri* detection per trap of the entire properties E, A and D was 0.016 (min 0.000 – max 0.146), 0.016 (min 0.000 and max 0.097) and 0.010 (min 0.00 and max 0.043), respectively. Properties didn’t differ (*p*≥0.05) on average *D. citri*/*trap*.

KDE maps demonstrated higher concentration of HLB eradicated trees in the peripheral areas of the properties (Figure 4). The higher concentrations of eradicated trees seem to be related with external areas, but it was also possible to observe a higher density of eradicated trees near internal roads or areas without citrus trees, indicating the importance of these areas to vector attraction.

**Figure 4.** KDE maps of citrus blocks from property E. Citrus blocks planted in 2012 with maximum density of 0.002 (a) and planted in 2009 with maximum density of 0.007 (b). Different colors correspond to ranges of HLB incidence according to the bars on the right side. Red dashed line and lilac area represent property periphery and landing strip, respectively.
3.4 Discussion

The results here presented give strong indications that primary spread from surrounding areas was predominant on the 24 studied citrus blocks (total area of 537.4 ha) under strict HLB management. Results of the modified Ripley’s K method and KDE maps demonstrated very short distances of weak spatial dependency between infected trees (average of 58.0 m) occurring in the border area of the citrus block. For most of the distances analyzed, Ripley’s K-function indicated, predominantly, a regular distribution of HLB eradicated trees, except for block 204, which presented aggregation distribution for all the distance analyzed (221.05). These results were supported by the higher averages of D. citri / trap and RSD distances found in blocks next to external areas rather than in blocks surrounded by citrus, despite the very low averages found. The spatial pattern observed and the strict HLB management performed in the areas indicates that the acquisition and transmission of the bacterium in trees within the orchard, i.e., the secondary spread should be rarely occurring. Results also demonstrated that the infective vectors flying from external inoculum sources (primary spread) land preferably in the first dozen of meters of the citrus blocks. After landing, the infective vector may infect one or a few trees before being killed by the frequent insecticide sprays. The predominance of the primary spread in the studied areas explains why aggregation was not found inside the citrus blocks.

Ripley’s K-function was used by Gottwald et al. (2010) in the study of HLB spatial point pattern in the Southern Gardens citrus groves (>4,800 ha), in South Florida. The frequency of insecticide sprays applied in Florida, at the time, was 6 times per year (Irey et al., 2008), while in the properties studied, at least 24 sprays per year. Very different from the results here presented, those authors verified a HLB pattern highly spatially related over large distance, with RSD between infected trees in an average distance of 3.5 km. Also, via a stochastic model, they observed that HLB spread occurred as an incessant mixture of primary and secondary infections, i.e., as a continuous introduction of inoculum from outside the area and simultaneous local spread within the area. According to the authors, the level of psyllid population was unprecedented high compared to other recorded psyllid infestations. Thus, the low frequency of psyllid management added to the high vector infestation may have favored the occurrence of secondary infections and, consequently, the strong RSD at very long distances found in Florida when compared to the results found in São Paulo.

In the same study, Gottwald et al. (2010) verified the most common distance between pairs of HLB-infected trees (MAX), with a median distance of 1.58 km. These results suggested that there was a spatial relationship that was repeated most frequently at about 1.58 km, and may well indicate a common distance for psyllid dispersal of HLB. Here, we observed MAX distance of 38.1 and 51.0 m
for blocks located internally and at the periphery of the properties, respectively. As these distances were related to the periphery area of the blocks by the KDE maps, they may indicate the most common distance for psyllid landing in a tree after entering the area, i.e., the range in which the most insects occurred.

HLB and *D. citri* higher incidences in peripheral areas receive the name of edge effect, and has been observed by many authors (Bassanezi *et al*., 2013; Bergamin Filho *et al*., 2016; Boina *et al*., 2009; Monteiro *et al*., 2013; Sétamou *et al*., 2015; Sétamou & Bartels, 2015; Tomaseto *et al*., 2015). *D. citri* behavior of frequent dispersion among groves also has been related to the spatial process behind the edge effects (Boina *et al*., 2009; Gottwald, 2010; Hall & Hentz, 2011). According to Sétamou & Bartels (2015), *D. citri* migration to, preferably, peripheral trees can be related to inter-generational population increase in new flush cycles, the result of migration processes, or differential vector performance on border areas. Regardless of the reasons of *D. citri* preferences for trees near the edge of groves, the edge effect is a strong indication of infective psyllid migration from surrounding areas, and, consequently, of HLB primary infections. Primary infections are responsible for introduction of HLB into new areas and are extremely difficult to prevent, and this is the biggest challenge in managing HLB in areas with strict management, i.e., HLB-tree eradication and vector control (Gottwald *et al*., 2010).

Besides demonstrating the concentration of infected trees mainly in the periphery area of the properties, with KDE we also demonstrated this pattern in the border area of internal citrus blocks that were 200 m away from external areas. This result indicates that the “cloud” of vectors, after departing from an external inoculum source, will preferably land in the first dozen of meters of the first citrus blocks of the property. However, a portion of the population will continue to move into the property and, in turn, will land preferentially near the roads, lamps or facilities. The tendency of HLB infections to concentrate near areas where citrus blocks face internal voids related to roads, canals, containers, houses, buildings and equipment was related by some authors (Bassanezi *et al*., 2005; Gottwald, 2008; Gasparoto *et al*., 2018). The higher density of eradicated trees occurring near the contrast between citrus and non-citrus areas indicates the importance of these areas to HLB new infections. Also, in the 24 blocks studies, the roads could be acting as a direct link between external areas and the interior of the properties.

Under the conditions in which this study was carried, a weak aggregation of eradicated trees was found at average distances of 58.0 m in the border of the citrus blocks, and a regular distribution prevailed in the areas. These results are indicating that primary spread prevails and the population of *D. citri* will land preferably in the first dozen of meters of the property, but a portion of the vector population will continue to dilute into the property and tend to aggregate near areas of contrast
between citrus and non citrus. Thus, knowing this disease behavior and also the strict management applied in the area, some assumptions can be made related to the disease control strategies.

As a result of the edge effect, many citrus growers have been intuitively increasing the frequency of insecticide sprays on the edges of their properties in an attempt to reduce the number of HLB-diseased plants. This strategy was used in properties A and D, mainly, and for a short period as it wasn’t effective (Table 4). This border sprays are usually performed by skirting the periphery of the grove with the spraying equipment facing the trees in the edge, without entering the grove. Producers believe that these border sprays would reduce *D. citri* population and, consequently, disease incidences. However, Asato (2018) verified that border spray presents an effective coverage (≥ 30% of the canopy) only in the first trees on the border of the grove and additional border sprays in intervals of 7 and 14 days weren’t efficient to reduce *D. citri* population and HLB symptoms. Based in the results here presents, we believe that even if applied in longer distances, the intensification of insecticide sprays in the border may not be effective to reduce HLB incidences. At first, some of the psyllids that land in the border of the citrus blocks are still able to cause infection before being killed by the insecticide. Then, a proportion of the *D. citri* “cloud” dilute into the property and promote new infections in internal blocks.

According to the founds evidencing the prevalence of primary spread, the main strategy that would effectively reduce HLB incidences in a property already performing a strict internal management, is an external reduction of inoculum sources. Strategies to reduce vector entering on the property, such as the non-eradication of peripheral trees, attractive killer plants or planting parallel to the roads could also help on HLB incidences reduction, but further investigations are needed. The importance of the *D. citri* “cloud” reduction is evidenced by the incidences found in the young citrus blocks here studied (6 to 9 years) under a strict HLB management. The 24 citrus blocks from properties E, A and D had, until 2017, averages of 2.4%, 4.5% and 14.0% of the trees eradicated, respectively. Eradication of HLB symptomatic trees doesn’t only mean loss of production, but also expenses with inspections by professional scouts, eradication operations, removal of the eradicated trees and replanting of healthy nursery trees. Furthermore, knowing of the higher incidence of HLB in the periphery of the properties and that contrast areas are more subjected to the occurrence of the disease, planning the planting of new orchard avoiding narrow shaped groves and contrasting areas between the blocks can be considered on orchards renovation.

Average *D. citri*/trap observed in properties E, A and D (0.016, 0.016 and 0.010, respectively), demonstrated a lower vector population compared with studies conducted in other countries and also in Brazil. Hall *et al.* (2008) reported a mean number of 6 and 10 *D. citri*/trap per week in the young and mature trees, respectively, in Florida. In Brazil, Bassanezi *et al.* (2013b) observed 0.08 to
0.46 adults per trap. The frequent insecticide sprays for psyllid control made in São Paulo-Brazil may partially explain the lower *D. citri* population densities in commercial citrus groves.

Different of higher RSD distances and average *D. citri*/trap, peripheral blocks didn’t present HLB higher incidences than internal blocks. The low difference between these groups can be related to the intense disease management performed in all the citrus blocks. This also can be related to the very high HLB incidences observed in the blocks of property D, when compared with properties E and A. Property D presented higher incidences in internal than peripheral blocks. Both, property D higher incidence when compared to the other properties and the higher incidences observed in internal blocks, may be related to the exposure of the healthy nursery trees to highly infected citrus blocks, before being planted and receive chemical management, and to the large interval of planting between the citrus blocks (Table 1).

The results here presented highlight the importance of the primary spread to the HLB epidemic and, consequently, the need of external management. Bassanezi *et al.* (2013b) have already demonstrated that an area-wide inoculum reduction and vector control is need for the reduction of the primary spread. Not only properties with poor or no HLB management as also the infected hosts in sidewalks and backyards have been a major problem for efficient HLB management (Bassanezi *et al.*, 2013a,b; Bové, 2014; Michigami *et al.*, 2015) and so, these plants should be included on the regional management. Perform an effective reduction of inoculum sources, including non-commercial citrus plants, is a challenge for HLB management in all America continent.

Despite the complexity for HLB management, the increasing disease incidences and the concerning situation of the agribusiness chain of citrus, relatively few spatial epidemiology studies have been conducted about the HLB pathosystem. This study shows, for the first time, the use of Ripley’s K- function method for spatial analysis of HLB epidemics in area under frequent HLB-tree eradication and vector control. By the very low *D. citri* populations identified in the yellow stick traps, we demonstrate the effectiveness of the vector control. However, even under these conditions, the properties suffered increase in HLB incidences and had a high amount of trees eradicated due HLB infection. To reduce HLB incidences, citrus groves must keep the strict management in all property area, but implement strategies that reduce *D. citri* entering the property. Thus, *D. citri* frequent monitoring is essential in the indication of external inoculum sources. In addition, efforts must be adopted to reduce the number of citrus and *Murraya* spp. plants maintained without vector control around commercial citrus areas.
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4. TEMPORAL PROGRESS OF HUANGLONGBING (HLB) AND SPATIAL DISTRIBUTION OF HLB AFFECTED AREAS AND THE VECTOR DIAPHORINA CITRI (HEMIPTERA: LIVIIDAE)

_Huanglongbing_ (HLB) is the main citrus disease and despite many efforts to its control disease incidence continues to increase in São Paulo State, Brazil. The objectives of this study were to better understand HLB epidemics under strict disease management and to demonstrate the importance of external sources of inoculum (primary spread) in HLB epidemics. Our study was carried out in a large commercial citrus property (~8,000 ha, 314 citrus blocks) located in the central area of São Paulo. This property performed strict HLB management (frequent symptomatic trees eradication and vector control). Gompertz and logistic temporal models were fitted to annual disease progress of 177 citrus blocks and average _Diaphorina citri_ (ACP) per trap were determined in 296 citrus blocks. Disease progress rate, HLB final incidence and average ACP per trap were compared between blocks located at the periphery (edge) or internally (surrounded by citrus) in the property. The findings, then, were related to external HLB inoculum sources. Both, Gompertz and logistic temporal models fitted well to HLB annual incidences, but Gompertz was chosen as the best model to describe HLB epidemics (higher $R^2$ and better distribution of residues). Values of Gompertz progress rates (ranging from 0.04 to 0.28 per year), average HLB incidence (0.11), and average ACP per trap (0.016) were lower compared with data in the literature. The variables differed ($p<0.05$) between peripheral and internal blocks, higher values being related mainly with blocks in the property’s periphery. A great amount of noncommercial trees potentially serving as HLB primary inoculum was found near citrus blocks presenting higher rates of disease progress, HLB incidences, and ACP per trap. The regional management of HLB should include, besides commercial orchards, noncommercial trees.

**Keywords:** Gompertz model; Logistic model; Regional management; External inoculum sources; Noncommercial plants

4.1 Introduction

_Huanglongbing_ (HLB) is a citrus vector disease associated with three phloem-restricted bacteria ‘*Candidatus* Liberibacters pp’. In America and Asia continents, epidemics of HLB are associated with ‘*Ca. Liberibacter asiaticus*’ (Clas). In São Paulo State (SPS) - Brazil, also occurs ‘*Ca. Liberibacter americanus*’ (Clam), but with an extremely low frequency compared to Clas. Those bacteria are transmitted by the Asian citrus psyllid (ACP), _Diaphorina citri_ (Kuwayama) (Hemiptera: Liviidae). The symptoms associated to HLB include yellow branches sparsely foliated, tree decline, asymmetric and ill-flavored fruits, aborted seeds and fruit drop. There aren’t, until now, resistant commercial varieties or curative methods for disease control and the prevention of new infections is
the most efficient way to reduce HLB effects. This preventive management started to be adopted in SPS as soon as HLB was identified, in March 2004 (Coletta Filho et al., 2004; Teixeira et al., 2005), and consist of three practices: the use of healthy plants, produced in insect-proof screen-houses; eradication of symptomatic trees; and chemical control of the vector, *D. citri* (Bové, 2006).

HLB account with a long incubation period (from months until years) and disease symptoms can be similar to those associated to nutritional deficiency or other diseases. Thus, for more efficient detection of symptomatic trees, inspections are indicated to be performed by trained and experienced team and with the use of platforms (Belasque et al., 2009). Even so, according to Bové (2014), only 60% to 70% of symptomatic trees are detected and those affected, but still asymptomatic, remain as sources of inoculum in the area under strict management. In addition, symptomatic trees detection and eradication are highly cost and, nowadays, it isn’t adopted by several growers in SPS.

On the other hand, the chemical control of the vector is a less costly and more accepted practice by the growers. Generally, in SPS, one to four insecticide applications per month are performed in commercial orchards. The vector population is monitored by yellow stick cards (traps) and/or visual inspection of leaves to help growers on the decision about the time and frequency of insecticide sprays (Bassanezi et al., 2013a,b), however the adoption of a fixed calendar schedule is the current practice. There is also a new tool available to help citrus growers on the *D. citri* population control called “Phytosanitary Warning System”, developed by a citrus growers association (Fundecitrus, 2018b). This system is based on the counts of thousands of yellow stick cards present in commercial and noncommercial areas in SPS. The population dynamics of adults, and its spatial distribution, are available in a website and warning messages are sent to citrus growers when the vector population increases in a given area. The practical effect of this system are insecticide sprays been applied at the same moment by neighboring citrus growers.

Since the first detection of HLB in SPS, in 2004, citrus growers have eradicated more than 40 million HLB symptomatic trees (CDA, 2017). This amount corresponds to more than 20% of the sweet orange (*Citrus sinensis* L.) trees population in the Citrus Belt area, main orange juice producer region in Brazil (Fundecitrus, 2018a). In addition, in 2018 it was estimated that 35.3 million orange trees (18.15%) were currently HLB symptomatic in this region (Fundecitrus, 2018a). Even carrying a strict management with frequent HLB-symptomatic trees eradication and vector control, new frequent HLB infections occur in any affected farm present in SPS in Brazil. By consequence, more than a thousand of citrus properties are no longer cultivated with sweet oranges since 2015, especially small- and medium-size properties (those with less than fifty thousand trees) (Fundecitrus, 2018a).
D. citri is able to disperse several kilometers without wind assistance (Martini et al., 2013, 2014; Lewis-Rosenblum et al., 2015) and can move frequently from abandoned to managed orchards (Boina et al., 2009; Tiwari et al., 2010; Lewis-Rosenblum et al., 2015). As presented by Bergamin Filho et al. (2016), in citrus farms maintained with strict management, new HLB infections occur mainly as a result of the dispersion of infective adults from external sources of inoculum, i.e., primary spread. The frequent HLB-symptomatic trees eradication and the low populations of D. citri inside of the managed properties, as result of frequent insecticide sprays, prevent the occurrence of new infections from HLB-affected trees present in the area under control, i.e., secondary spread. However, nymphs and adults can acquire the bacterium from infected plants present in commercial properties with poor management, abandoned areas, backyards or sidewalks, and migrate to orchards under strict management, and then transmit the bacteria to healthy trees before being reached by chemical control.

As a consequence of the primary spread from neighboring properties that don’t perform HLB management, most of HLB-eradicated trees are on orchard’s edges (edge effect) (Gottwald et al., 2007; Gottwald et al., 2008; Monteiro et al., 2013; Shen et al., 2013; Sétamou & Bartels, 2015; Tomaseto et al., 2015; Chinelato, 2017). Monteiro et al. (2013) and Chinelato (2017) showed that 80% of HLB-eradicated trees were in the first 80-160 m from the border of the orchard. Gasparoto et al. (2018) found higher values of HLB incidences and rates of disease annual progress at the citrus blocks closer to the edge than those located around inner sections of the farm. Therefore, for successful HLB management, the removal of symptomatic trees and psyllid control must be accomplished regionally by citrus growers (Bassanezi et al., 2013b; Bergamin Filho et al., 2016). Further than a regional management performed by neighboring commercial properties, noncommercial citrus trees and orange jasmine (Murraya spp.), an ornamental plant frequently used in Brazil and an alternative host for D. citri and ‘Ca. Liberibacter spp.’, also have to be a target on a program of inoculum sources reduction. These plants are usually present in residences, sidewalks, swamps, woods and cattle fields and do not receive any chemical treatment for vector control as they are often unnoticed as important sources of inoculum.

Many studies have demonstrated the higher occurrence of HLB and D. citri on the periphery of the properties. This behavior was associated to external sources of inoculum and HLB primary spread (Bergamin Filho et al., 2016). However, no studies have demonstrated the HLB incidences, its temporal progress, and D. citri distribution in several orchards maintained under strict HLB management and its relationship with commercial and noncommercial neighboring areas. These are the objectives of the present study. We hypothesize that HLB-infected citrus and orange jasmine plants, maintained without insecticide sprays around citrus properties in SPS, are the sources of
inoculum for the primary spread of HLB in managed areas. By consequence, a more effective HLB management could be achieved with the eradication of those noncommercial sources of inoculum.

4.2 Material and Methods

4.2.1 Area

HLB was studied in a large commercial citrus property (~8,000 ha), that performed strict HLB management, located in the central area of SPS, Brazil (Figure 5). The property was composed of 314 rectangular citrus blocks, which ranged from 0.6 to 41.2 ha (average size of 21.0 ha). First, HLB and *D. citri* were studied in 177 citrus blocks distributed in the property and separated in peripheral (85 blocks) and internal (92 blocks) (Figure 5A). The next approach was to study HLB and *D. citri* in five groups of citrus blocks uniform in age and variety, located at different areas of the property, and also separated in peripheral and internal blocks (Figure 5B and Table 6). Finally, the findings on HLB and *D. citri* distribution in the property were compared with external HLB inoculum sources found by the surveys conducted by employs of the property.

Figure 5. Commercial citrus property composed of 314 citrus blocks of which 177 were separated in peripheral (blue) and internal (orange) (A) and 144 were separated into five groups, uniform in age and varieties: 1985-86 (orange), 1993-94 (yellow), 1997 (green), 2001 (red) and 2009 (blue) (B). Darker and lighter colors represent peripheral and internal blocks, respectively. White square, inside the farm, on northwest side.
represent a permanent preservation area. Citrus blocks surrounded by citrus were considered to be internal and citrus blocks with at least one side facing the property external area or the permanent preservation area were considered to be peripheral.

**Table 6.** Detailed information about the citrus blocks groups 1985-86, 1993-94, 1997, 2001 and 2009. Number of citrus blocks, number of internal and peripheral citrus blocks, varieties of scion and rootstock and total area of each group are presented.

<table>
<thead>
<tr>
<th>Group</th>
<th>Citrus blocks</th>
<th>Internal/external Citrus blocks</th>
<th>Scion/rootstocks</th>
<th>Area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985-86</td>
<td>25</td>
<td>19/6</td>
<td>Natal x Rangpur lime</td>
<td>589.3</td>
</tr>
<tr>
<td>1993-94</td>
<td>51</td>
<td>26/25</td>
<td>Valência Argentina x Rangpur lime</td>
<td>1,124.1</td>
</tr>
<tr>
<td>1997</td>
<td>31</td>
<td>13/18</td>
<td>Valência Argentina x Rangpur lime</td>
<td>591.3</td>
</tr>
<tr>
<td>2001</td>
<td>9</td>
<td>3/6</td>
<td>Natal x Rangpur lime</td>
<td>190.0</td>
</tr>
<tr>
<td>2009</td>
<td>28</td>
<td>21/7</td>
<td>Pera Rio x Sunki mandarin (19) Hamlin x CitromeloSwingle (8)</td>
<td>619.0</td>
</tr>
</tbody>
</table>

**4.2.2 HLB management**

The studied property performed a strict management for HLB, with frequent inspections and eradication of symptomatic trees, monitoring and frequent insecticide sprays for *D. citri* control and the use of healthy plants for new citrus blocks planting or replanting of eradicated trees. From 2014, regional management was carried, by the property’s employs, for inoculum sources reduction neighboring the property.

Surveys for HLB symptomatic trees were performed in every tree of each citrus block, at least four times per years, from 2007, by a professional team. From 2012, the surveys of adult blocks (>3 years old) counted with the use of tractor-pulled platforms to assist in the detection of HLB symptom (Belasque *et al.*, 2010). Confirmation of infection was made by PCR analysis when the symptomatology wasn’t conclusive. Symptomatic trees, when detected, were eradicated within 30 days.

Monitoring of ACP population was performed by visual inspections of branches end detection in traps. ACP visual inspections were performed at least twice a month, by a professional team, on 1% of all trees, three branches per tree of each block, for detection of eggs, nymphs or adults of *D. citri*. Detections by traps were performed by yellow adhesive traps of 10 x 30 cm (ISCA) fixed in the upper third of trees, placed on the periphery of the citrus blocks, at least one trap per block. Traps
were analyzed weekly, by a professional team, for detection of *D. citri* and evaluations were carried out directly in the field until Sep 2014, and, after, inside property’s facilities.

*D. citri* chemical control was performed with contact insecticides applied via ground, aerial or in border sprays. Ground sprays were carried with tractor and turbo sprayer (1,000 L/ha), at least twice a month. Frequency of sprays followed a risk scale: blocks up to two years received weekly sprays; blocks over two years received applications twice a month when located on the periphery of the property or near forest and marsh; and monthly when located internally. Ground sprays were intensified according to *D. citri* detection. Five to six aerial sprays were performed per years, by airplane (Ipanema, 5.0 L/ha - application range of 18 m), on the entire property based on the Phytosanitary alert recommendation (Fundecitrus, 2018b). Besides contact insecticide sprays, blocks up to two years received two systemic insecticide applications (neonicotinoids - 200 to 1000 mL/tree), via drench, every two months between Sep and Mar (period of higher *D. citri* population).

The regional management was performed, by employs of the property, from 2014 and counted on the execution of inspections on the external area, within a radius of 5 km from the edge of the property. Thus, inspection teams searched for noncommercial citrus or orange jasmine trees in swamps, woods, cattle fields, communities and backyards. When these trees were found, the eradication of the trees or the chemical control of the vector were negotiated with the owner.

Release of *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae), a natural enemy of *D. citri*, was also used for regional management.

### 4.2.3 Data analysis

First, HLB and *D. citri* were studied in 177 citrus blocks distributed in the property. Blocks were composed of different combinations of sweet oranges scions and rootstock and were planted between 1985 and 2009. These blocks were separated, for comparison, in peripheral and internal citrus blocks (85 and 92, respectively). The next approach was to study HLB and *D. citri* in peripheral and internal blocks of each of the five groups of citrus blocks (1985-86, 1993-94, 1997, 2001 and 2009), uniform in age and varieties, and located at different areas of the property. Finally, the findings on HLB and *D. citri* distribution in the property were discussed with external inoculum sources found by the regional management. Data for eradication of symptomatic trees (for 177 blocks), capture of *D. citri*/trap (of 296 blocks) and local, type and amount of external sources of inoculum were provided by the studied property, for this study.

Data of eradication of symptomatic trees (from the first eradication performed in each citrus block until 2014-2016) was used to calculate the cumulative disease proportion and the area under
the disease progress curve (AUDPC), for the 177 blocks. AUDPC was calculated standardized in time (AUDPC*) according to the equation cited by Campbell & Madden (1990). Cumulative annual proportion of HLB-eradicated trees was used to the fitting of the temporal models logistic \[ y=1/(1+((1/Y_0)-1)*\exp(-r*t)) \] and Gompertz \[ y=\exp(-\{\ln(Y_0)\exp(-r*t)\}) \], by linear regression. For equations, \( y \) is the proportion of eradicated trees at the time \( t \), \( Y_0 \) is a parameter related to the initial inoculum and \( r \) is the estimated disease progress rate. The fitting of the temporal models to cumulative annual proportion of HLB was evaluated by the adjusted coefficient of determination (\( R^2* \)) and the distribution of the residues. The number of trees eradicated in the first eradication of each citrus block was considered to be the real initial inoculum (real \( Y_0 \)), despite the variable incubation period of HLB.

Data of capture of \( D. \) citri/trap (from Jun 2012 until Dec 2016), for the 296 citrus blocks, was used to calculate the average \( D. \) citri/trap of each block (considering four traps evaluation per month) and the population dynamics of the vector. The HLB incidence, AUDPC*, rate of disease progress (\( r \)), real \( Y_0 \) and average \( D. \) citri/trap were compared, by the non-parametric test of Kruskal-Wallis, between the peripheral and internal blocks of the 177 citrus blocks and the five uniform groups (1985-86, 1993-94, 1997, 2001 and 2009). These same variables plus the disease incidence estimated by the temporal model were correlated, by the non-parametric Spearman's rank-order correlation, between the 177 citrus blocks. The distribution of the variables HLB incidence, AUDPC*, rate of disease progress (\( r \)) and average \( D. \) citri/trap in the property, and the external inoculum sources of HLB found on a 5 km radius regional management, were presented in QGIS maps (Graduated style).

4.3 Results

Temporal analyses indicated that the Gompertz and logistic models fitted well to HLB annual incidence for the epidemic occurring under intense management in central SPS. Both models fitted to the 177 citrus blocks studied (\( p<0.05 \)). Average adjusted \( R^2 \) (\( R^2* \)) was 0.95 (ranging from 0.78 to 0.99) and 0.91 (ranging from 0.62 to 0.99) for the Gompertz and logistic models, respectively. Besides presenting \( R^2* \) generally superior, Gompertz model also presented better distribution of the residues and was chosen as the best temporal model to describe HLB epidemic in the conditions this study was performed. Gompertz model, fitted to HLB annual progress of 8 citrus blocks, and the respective rate of disease progress (\( r_G \)) and \( R^2* \) are presented in Figure 6.
Figure 6. The Gompertz temporal model (black line) fitted to HLB annual proportion (circles) in 8 of the 177 citrus blocks tested. Respective rates of disease progress ($r_G$) and adjusted $R^2$ ($R^2*$) are presented.

HLB Incidence, AUDPC*, $r_G$ and average $D. \ citri$/trap differed ($p \leq 0.05$), for the 177 citrus blocks, between blocks located at the periphery or internally at the property (Table 7). However, the difference wasn’t so great for $r_G$ (0.125 vs. 0.139, $p=0.04$) and there wasn’t difference for real $Y_0$ between the location of the citrus blocks in the property. The slopes of the regression lines, i.e., the $r_G$, for the 177 citrus blocks, presented an average of 0.13 (ranging from 0.04 to 0.27) and 98 citrus blocks (55%) presented $r_G \leq 0.13$ (of which 59 were intern and 39 were peripheral). For the logistic
temporal model, the average rate was 0.52 (ranging from 0.14 to 1.22). Average HLB Incidence, AUDPC*, real $Y_0$ and $D.\ citri/\text{trap}$, for the 177 citrus blocks, was 0.114, 0.046, 0.005 and 0.016, respectively.

Table 7. Average, median, minimum (Min) and maximum (Max) HLB incidence, AUDPC*, rate of disease progress ($r_G$), real $Y_0$ and average $D.\ citri/\text{trap}$, of the 177 citrus blocks separated in peripheral and internal blocks. $p$-values resulted for the comparison between intern and peripheral blocks are presented.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Average</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incidence</strong></td>
<td>0.094</td>
<td>0.081</td>
<td>0.005</td>
<td>0.277</td>
<td>0.135</td>
<td>0.111</td>
<td>0.011</td>
<td>0.463</td>
<td>0.0004</td>
</tr>
<tr>
<td><strong>AUDPC</strong></td>
<td>0.042</td>
<td>0.032</td>
<td>0.003</td>
<td>0.127</td>
<td>0.051</td>
<td>0.047</td>
<td>0.003</td>
<td>0.137</td>
<td>0.0072</td>
</tr>
<tr>
<td>$r_G$</td>
<td>0.125</td>
<td>0.124</td>
<td>0.042</td>
<td>0.223</td>
<td>0.139</td>
<td>0.139</td>
<td>0.059</td>
<td>0.267</td>
<td>0.0446</td>
</tr>
<tr>
<td>$Y_0$</td>
<td>0.005</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.042</td>
<td>0.005</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>0.046</td>
<td>0.1294</td>
</tr>
<tr>
<td>$D.\ citri/\text{trap}$</td>
<td>0.008</td>
<td>0.004</td>
<td>0.000</td>
<td>0.054</td>
<td>0.025</td>
<td>0.021</td>
<td>0.000</td>
<td>0.096</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^1$Cumulative HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16

$^2$D. citri data from Jun 2012 until 2014-16

Maps of the distribution of HLB incidence, AUDPC*, $r_G$ (for 177 citrus blocks) and $D.\ citri/\text{trap}$ (for 314 citrus blocks) in the property are presented in Figure 7. HLB incidence and AUDPC* presented a very similar spatial distribution. Of the 25 highest incidences observed in the 177 blocks (>0.21 proportion of HLB-infected trees/block), 18 were located on the periphery of the property. For the average of $D.\ citri/\text{trap}$, 29 citrus blocks presented no detection on the period studied, of which 3 were peripheral and 26 were internal. The distribution of the variables (Figure 7) demonstrated that the higher values of HLB incidence, AUDPC*, $r_G$ and $D.\ citri/\text{trap}$ are commonly related to the periphery. However, higher values of the variables can also be seen in some internal blocks and lower values can be seen in peripheral blocks.
Figure 7. Maps of the distribution of HLB progress rate (obtained by the Gompertz model, $r$) (A), HLB incidence (in proportion) (B), AUDPC* (D) (for 177 citrus blocks) and average $D.\ citri$/trap (D) (for 296 citrus blocks). HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16. $D.\ citri$/trap data from Jun 2012 until 2014-16.

Statistically significant ($p \leq 0.05$) Spearman correlations were observed between the variables HLB incidence, real $Y_0$, HLB incidence estimated by the Gompertz model (Incidence G), rate of disease progress ($r_G$), AUDPC* and average $D.\ citri$/trap, except between real $Y_0$ and average $D.\ citri$/trap (Table 8). The strongest correlations ($>0.95$) were observed between HLB incidence, HLB incidence G
and AUDPC*. In general, the variable that presented the weakest correlations was the average *D. citri*/trap.

Table 8. Spearman correlations of HLB incidence, real *Y₀*, HLB incidence estimated by the Gompertz model (Incidence G), rate of disease progress (*r*G), AUDPC* and average *D. citri*/trap, for the 177 citrus blocks.

<table>
<thead>
<tr>
<th></th>
<th>Real <em>Y₀</em></th>
<th>Incidence G</th>
<th><em>r</em>G</th>
<th>AUDPC*¹</th>
<th>Average <em>D. citri</em>/trap²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence¹</td>
<td>0.59</td>
<td>0.98</td>
<td>0.36</td>
<td>0.95</td>
<td>0.30</td>
</tr>
<tr>
<td>Real <em>Y₀</em></td>
<td>0.58</td>
<td>−0.39</td>
<td>0.74</td>
<td>0.25</td>
<td>0.09¹</td>
</tr>
<tr>
<td>Incidence G</td>
<td></td>
<td>0.39</td>
<td>0.96</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td><em>r</em>G</td>
<td></td>
<td>0.19</td>
<td></td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>AUDPC*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
</tbody>
</table>

¹Variables with no correlation (p>0.05)

HLB incidence and AUDPC* data from citrus blocks since the first eradication of HLB-symptomatic trees until 2014-16

²*D. citri*/trap data from Jun 2012 until 2014-16

Average, minimum and maximum HLB incidence, AUDPC*, *r*G, real *Y₀*, and average *D. citri*/trap for the five uniform groups located at different areas of the property are presented in Table 9. Groups 1985-86, 1993-94, 1997 and 2001 presented higher averages of HLB Incidence, AUDPC* and real *Y₀* than the group 2009. On the other hand, average *r*G was very similar between the five groups (ranging between 0.12 and 0.14). For *D. citri*/trap, it was observed smaller averages of vector detected per trap on groups 2001 and 2009 rather than the older groups. For groups 1985-86, 1993-94, 1997, 2001 and 2009 it was observed 2, 11, 5, 2 and 5 blocks with no *D. citri* detection on the period studied. The difference between the cumulative incidences of the groups can be more clearly seen in Figure 8. Groups 1985-86, 1993-94, 1997, 2001 showed an increase in the incidence of HLB from 2011-12.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence¹</th>
<th>AUDPC*¹</th>
<th>rG</th>
<th>Real Y₀</th>
<th>D. citri²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Min - Max)</td>
<td>(Min - Max)</td>
<td>(Min - Max)</td>
<td>(Min - Max)</td>
<td>(Min - Max)</td>
</tr>
<tr>
<td>1985-86</td>
<td>0.137</td>
<td>0.061</td>
<td>0.143</td>
<td>0.005</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>(0.051 - 0.253)</td>
<td>(0.023 - 0.121)</td>
<td>(0.102 - 0.205)</td>
<td>(&lt;0.001 - 0.033)</td>
<td>(0.000 - 0.058)</td>
</tr>
<tr>
<td>1993-94</td>
<td>0.137</td>
<td>0.052</td>
<td>0.141</td>
<td>0.005</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>(0.041 - 0.277)</td>
<td>(0.015 - 0.127)</td>
<td>(0.087 - 0.232)</td>
<td>(&lt;0.001 - 0.046)</td>
<td>(0.000 - 0.096)</td>
</tr>
<tr>
<td>1997</td>
<td>0.135</td>
<td>0.058</td>
<td>0.116</td>
<td>0.007</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>(0.046 - 0.266)</td>
<td>(0.022 - 0.111)</td>
<td>(0.057 - 0.172)</td>
<td>(0.001 - 0.022)</td>
<td>(0.000 - 0.085)</td>
</tr>
<tr>
<td>2001</td>
<td>0.195</td>
<td>0.088</td>
<td>0.126</td>
<td>0.013</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>(0.057 - 0.304)</td>
<td>(0.030 - 0.137)</td>
<td>(0.053 - 0.166)</td>
<td>(0.002 - 0.036)</td>
<td>(0.000 - 0.021)</td>
</tr>
<tr>
<td>2009</td>
<td>0.026</td>
<td>0.010</td>
<td>0.136</td>
<td>0.001</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>(0.005 - 0.073)</td>
<td>(0.003 - 0.020)</td>
<td>(0.074 - 0.234)</td>
<td>(&lt;0.001 - 0.005)</td>
<td>(0.000 - 0.029)</td>
</tr>
</tbody>
</table>


The population dynamics of the vector in the property versus each of the five groups is presented in Figure 9. Although the group 2001 only showed detection after the second quarter of 2016, the groups 1985-86, 1993-94, 1997 and 2001 presented peaks of D. citri/trap higher than the mean average D. citri/trap of the entire property, different from group 2009. Generally, the D. citri/trap peaks were related to the period between Jul and Jan.

HLB Incidence, AUDPC*, rG, real \( Y_0 \) and average *D. citri*/trap were compared, for each of the five groups, between peripheral and intern citrus blocks (Table 10). For group 1985-86, the only difference \( (p \leq 0.01) \) was observed on the average of *D. citri*/trap, which was higher on peripheral blocks. The group 1993-94 presented higher \( (p \leq 0.01) \) HLB incidence, rG and average *D. citri*/trap on peripheral blocks. The group 1997 presented higher \( (p \leq 0.05) \) rG and average of *D. citri*/trap on peripheral blocks. The groups 2001 and 2009 didn’t differ \( (p > 0.05) \) in any of the variables compared between peripheral and internal citrus blocks.
Table 10. Average, minimum (Min) and maximum (Max) HLB incidence, AUDPC*, rG, real Y₀ and average D. citri/trap of peripheral and internal citrus blocks of the groups 1985-86, 1993-94, 1997, 2001 and 2009. p-values resulted for the comparison between intern and peripheral blocks are presented.

<table>
<thead>
<tr>
<th>Block</th>
<th>Incidence*1</th>
<th>AUDPC*1</th>
<th>rG</th>
<th>Real Y₀</th>
<th>Average D. citri/trap²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periphery 85-86</td>
<td>0.166</td>
<td>0.072</td>
<td>0.164</td>
<td>0.004</td>
<td>0.040</td>
</tr>
<tr>
<td>Intern 85-86</td>
<td>0.128</td>
<td>0.057</td>
<td>0.136</td>
<td>0.005</td>
<td>0.017</td>
</tr>
<tr>
<td>p-value 85-86</td>
<td>0.112</td>
<td>0.203</td>
<td>0.112</td>
<td>0.703</td>
<td>0.007</td>
</tr>
<tr>
<td>Periphery 93-94</td>
<td>0.163</td>
<td>0.056</td>
<td>0.155</td>
<td>0.005</td>
<td>0.029</td>
</tr>
<tr>
<td>Intern 93-94</td>
<td>0.113</td>
<td>0.048</td>
<td>0.126</td>
<td>0.004</td>
<td>0.010</td>
</tr>
<tr>
<td>p-value 93-94</td>
<td>0.014</td>
<td>0.127</td>
<td>&lt;0.001</td>
<td>0.888</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periphery 1997</td>
<td>0.143</td>
<td>0.059</td>
<td>0.126</td>
<td>0.005</td>
<td>0.037</td>
</tr>
<tr>
<td>Intern 1997</td>
<td>0.124</td>
<td>0.058</td>
<td>0.100</td>
<td>0.010</td>
<td>0.007</td>
</tr>
<tr>
<td>p-value 1997</td>
<td>0.317</td>
<td>0.749</td>
<td>0.028</td>
<td>0.215</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periphery 2001</td>
<td>0.197</td>
<td>0.082</td>
<td>0.138</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Intern 2001</td>
<td>0.191</td>
<td>0.100</td>
<td>0.101</td>
<td>0.022</td>
<td>0.003</td>
</tr>
<tr>
<td>p-value 2001</td>
<td>0.796</td>
<td>0.302</td>
<td>0.070</td>
<td>0.071</td>
<td>0.221</td>
</tr>
<tr>
<td>Periphery 2009</td>
<td>0.029</td>
<td>0.012</td>
<td>0.137</td>
<td>0.001</td>
<td>0.015</td>
</tr>
<tr>
<td>Intern 2009</td>
<td>0.025</td>
<td>0.010</td>
<td>0.136</td>
<td>0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>p-value 2009</td>
<td>0.212</td>
<td>0.326</td>
<td>0.771</td>
<td>0.770</td>
<td>0.209</td>
</tr>
</tbody>
</table>


Many potential external sources of inoculum, composed mainly by noncommercial citrus or orange jasmine trees, were found around the property (Figure 10 – green and yellow circles). These trees were distributed mainly to the north and west sides of the property. The largest quantities of external inoculum sources found in a single site (yellow circles) were located: in the northwestern area (totaling 3,050 trees) near the group 1997; in the northeast area (totaling 439 trees), near the group 1993-94; and in the southeast area of the property (totaling 7,544 trees), near the group 1985-86. Commercial properties that performed HLB management (trees eradication and insecticide sprays) were not considered to be external inoculum sources. Also, in addition to external sources of inoculum, many noncommercial and HLB-symptomatic trees or shoots were found within the property (red circles). These plants are mostly shoots in citrus blocks that were eradicated for reform and trees that grew in woods and swamps within the property. The internal sources of inoculum were mainly related to groups 1985-86, 1997 and 2001.
Higher HLB incidence and average *D. citri*/trap (Figure 10B and C) occurred near external (or non-external) sources of inoculum, as can be seen for the northwestern, northeast and southeast areas of the property. These areas presented the largest quantities of external inoculum sources found in a single site (yellow circles). However, in the central area of the property (the area composed by the group 2001), very high HLB incidences as well as external and internal sources of inoculum were found, but low *D. citri*/trap averages were observed.

![Figure 10.](image)

4.4 Discussion

Temporal analyses indicated that both the Gompertz and logistic temporal models fitted well to HLB annual incidences for the epidemic occurring under intense management in central SPS. According to Bergamin Filho *et al.* (2008), the fitting of both models to HLB disease progress is
related to the lack of asymptotic data, i.e., data in which disease incidence approaches the highest level. This fact, in turn, is related to the impossibility of maintaining orchards with high incidence of the disease, since they will be economically unviable long before the incidence reaches 100%. Due presenting better distribution of residues and higher $R^2*$ than the logistic, Gompertz was chosen as the temporal model that best described the HLB epidemic studied.

Even HLB being a multiyear disease (polyetic), given the perennial nature of citrus and comparing the epidemic with other arboreal diseases, HLB is considered to increase rapidly (Gottwald, 2010a). The rapid rate of HLB increase may be associated with the presence of large amounts of inoculum and high population of the vector. Characteristics of the HLB, such as the short latent period and the long incubation period difficult the identification of inoculum sources and disease control. Furthermore, the rapid distribution of the pathogen in the plant, although not regular, may also be associated with the fast rates of disease progression.

However, the HLB progress rates estimated by the Gompertz ($r_G= 0.04$ to $0.28$) and logistic ($r_L= 0.14$ to $1.22$) models here presented were lower than most of the progress rates for HLB published in literature. Rates of the annual progress of a HLB epidemic, studied in a large area in south Florida - USA, estimated by the Gompertz model, ranged from 0.44 to 0.97, while logistic rates varied from 0.72 to 2.73 (Gottwald et al., 2010b). In HLB epidemics studied in Brazil, in 2013, under conditions of strict management, logistic rates varied from 0.82 to 2.77 (Bassanezi et al., 2013a,b). Recently, also in Brazil, HLB annual progress rates estimated by the Gompertz model ranged from 0.10 to 0.40 for 85% of citrus blocks in conditions similar to those of this study, with strict disease management in a large property (Gasparoto et al., 2018). However, even in this last case in which the conditions were similar, only a few rates were smaller than $r_G= 0.13$, as the 98 blocks (55% of 177 blocks) of this study. Temporal epidemiological parameters of HLB, such as $r_G$, are dependent on inoculum sources, vector populations, age of the orchard and numerous environmental factors (Bassanezi & Gottwald, 2009; Gottwald, 2010a) and may, together with the disease management differences, explain the variation of HLB progress rates.

Higher HLB incidences and annual disease progress rates were observed at peripheral citrus blocks by Gasparoto et al. (2018). Those and other previous authors related this to the vector migration from external sources of inoculum and emphasized the need for stricter disease control at these areas as well the need to extend the control to external sources of inoculum. When $r_G$, HLB Incidence, initial inoculum (real $Y_0$), AUDPC* and average $D. citri$/trap, of the 177 citrus blocks, were compared between citrus blocks located at the periphery or internally, higher values were found in blocks located at the periphery of the property, except for real $Y_0$. Even $r_G$ being little variable, it was higher in peripheral (0.139) than in internal (0.125) citrus blocks. These results were consistent with
the maps of distribution of HLB incidence, AUDPC* and \textit{D. citri}/trap, which were more clearly associated with peripheral blocks than \textit{rG}.

Peripheral and internal citrus blocks didn’t differ in the variable real \( Y_0 \). Model fitting of mathematical functions, such as the Gompertz and logistic, to temporal data of epidemics, is highly dependent of the real \( Y_0 \). Small variations at the parameter \( Y_0 \) can result in high variations at the rate of disease progress (\( r \)). As presented in Table 8, real \( Y_0 \) correlated negatively with \textit{rG} (Spearman= –0.39, \textit{p}-value= 0.05). By consequence, HLB epidemics showing similar temporal rates (\( r \)) can differ about the real \( Y_0 \), HLB final incidence and AUDPC*. For example, the citrus blocks that presented the highest incidences of removed trees in our study, weren’t, necessarily, those with the highest \textit{rG} or \textit{rL}, the opposite being also true. The last two graphs of Figure 6 presented \textit{rG}= 0.20, but HLB incidences were 0.25 and 0.10, reached in 7 years (between 2007 and 2014). Both blocks were planted in 1985, but varied in real \( Y_0 \) and location, which were 0.0036 (peripheral, 0.25 HLB incidence) and 0.0003 (internal, 0.10 HLB incidence). As presented at Table 8, HLB incidences, real \( Y_0 \) and AUDPC* presented higher correlation between each other than with \textit{rG}. These results indicate that HLB epidemics cannot be compared only by temporal progress models. HLB final incidences and AUDPC*, as observed here, can be more useful to compare HLB-affected areas than only annual progress rates adjusted by temporal models. The calculation of the AUDPC* allowed a more adequate comparison of HLB incidences between blocks with different disease evaluation period. However, even with incidences of HLB having been evaluated in different periods for each block, HLB Incidence and AUDPC* presented a very similar spatial distribution at the property and a high Spearman correlation (0.95).

The average of \textit{D. citri}, for the 177 citrus blocks, was 0.016 \textit{D. citri}/trap (the same average of 0.016 was observed for the 296 citrus blocks) and it’s a very low average compared to the presented in the literature. Even the average \textit{D. citri}/trap observed in peripheral blocks is considered low (0.025). In Florida, mean number of 6 and 10 \textit{D. citri}/trap were reported by Hall \textit{et al.} (2008). In Brazil, Bassanezi \textit{et al.} (2013b) observed 0.08 to 0.46 adults per trap. The strict HLB management and the large size of the property may partially explain the lower \textit{D. citri} population densities in a commercial citrus grove. In relation to the population dynamics of \textit{D. citri}, the vector peaks were observed in the periods between Jul and Jan of the following year. Similar observations were made in SPS by Yamamoto \textit{et al.} (2001), Leal (2009) and Santos (2013). The authors related the peaks with the higher temperatures, air humidity, and intense emission of vegetative flushes in citrus trees at the spring season.

The average \textit{D. citri}/trap was the variable that differentiated more between internal (0.008) and peripheral (0.025) citrus blocks. This result corroborates with Sétamou & Bartels (2015), who observed a higher population density of the vector in the fields located in the border of the property.
Even so, in our study, some blocks of periphery presented average *D. citri*/trap equal to internal blocks or equal to zero. This difference is related to the predominance of the HLB primary dissemination and to the long-distance vector dispersion. Thus, peripheral blocks are more subject to external sources of inoculum, despite the strict management adopted in some farms of SPS. This also explains absence of vector detection, in the period studied, for some of the peripheral blocks, since they are at the periphery but might not be close to external sources of inoculum.

In general, the variable average *D. citri*/trap presented the weakest correlations. Other variables, such as HLB incidence, are more influenced by the strict management of the disease, performed in the property. Since the management is not efficient against the migration of infective *D. citri* from external plants and farms, this variable is more affected by the location of the blocks (peripheral or internally) than the local vector control. This explains the complexity of HLB management, compared to other diseases, since the disease can be, as in the case presented here, more dependent of external sources of inoculum.

Higher averages of HLB incidence, AUDPC* and real\(Y_0\) were found in groups 1985-86, 1993-94, 1997 and 2001 than in the group 2009 (Table 9 and Figure 8). It was also possible to observe a higher increase in disease progress in the years 2011-12, the same years that platforms for inspection for symptomatic HLB trees were implemented. Platforms allowed a higher HLB-symptomatic tree detection than walking inspections (Belasque et al., 2009). Groups1985-86, 1993-94, 1997 and 2001 also presented peaks of *D. citri*/trap higher than the mean average of the property, different from group 2009. The 2001 group present almost all blocks facing external areas of the property, pattern not existing for 2009 group. In addition, trees at the 2009 group were cultivated in a period with more knowledge generated about HLB and vector spread, especially for vector control.

All groups showed few differences between internal and peripheral blocks. This can be explained by the small variation of the variables within each group. The variable that most differed was average *D. citri*/trap (differed for groups 1985-86, 1993-94 and 1997). However, despite the differences in the other variables, \(r_G\) was very similar (ranging between 0.12 and 0.14) between the five groups. It is interesting to note blocks of trees with different ages, varieties, located in different places in a very large area, presenting very similar disease progress rates. As discussed previously in this study, temporal models as Gompertz and logistic are not adequate to capture the primary spread that governs the epidemics in areas under strict management. Though these models can be used to compare HLB epidemics, some other variables are necessary to a better description of the disease pattern.

The findings on HLB and *D. citri* distribution inside the property were compared with the external HLB inoculum sources found by the surveys conducted by employs of the property (Figure 10). Those surveys started in 2014, but were intensified after that. The objective was to detect and
remove possible sources of inoculum of HLB/vector. A great number of noncommercial external sources of inoculum was found around the property, and seemed to be in accordance to the HLB incidence and average D. citri/trap spatial distribution. In addition, many plants that could serve as source of inoculum for HLB were found even within the property. It was not possible to analyze the HLB epidemics of all block of the property. However, the spatial distributions of the average D. citri/trap in the blocks and the potential inoculum sources localized inside and outside of the property are very useful to establish the probable relationship between noncommercial plants, i.e., plants not sprayed with insecticides for vector control, and the HLB infections occurred in the studied blocks.

HLB Incidences, average D. citri/trap and the rG found in this study were low compared to other HLB epidemics described in the literature. This must be related to the intense management of the disease carried out in the studied property and also to the large size of that property. The highest values of HLB incidences and average D. citri/trap were found mainly in peripheral blocks, demonstrating, in turn, the influence of external sources of inoculum to the HLB epidemic. In addition, those blocks were in the proximity of sites where most external potential sources of inoculum were found. Thus, yellow stick traps are useful in SPS conditions not only to a better vector control inside of managed areas, but also to identify locations where noncommercial plants could be present around the property.

In the search for ways to reduce the devastating effects caused by HLB in the America’s citriculture, many changes are and still will be made to turn the citrus production economically viable. According to da Graça et al. (2016), keep an economic production of citrus is the largest challenge ever faced by the citrus industry worldwide. The current scenario involves a lot of resources investment by government and citrus industry in researches focusing in finding and implementing strategies to minimize the infection and the symptoms progress on citrus trees. HLB is a complex disease and the management adopted inside the orchards aren’t effective in reducing disease incidences. As justified by Bassanezi et al. (2013b), it is necessary to manage the disease regionally. However, as nowadays conducted in important citrus producing areas in America (SPS, other regions in Brazil, Florida, Mexico), the “regional management” adopted by commercial growers corresponds to only insecticide sprays applied in a short period by neighboring farms. As demonstrated here, low levels of vector population and HLB incidences are achievable, in large areas in SPS, when the three practices for HLB management are adopted. In addition, but not less important, as presented here, HLB epidemics occurs by primary spread in managed areas in SPS and noncommercial citrus and orange jasmine plants are the mainly sources of HLB inoculum.

By conclusion, better HLB management could be achieved by the eradication of noncommercial citrus and orange jasmine plants maintained without insecticides sprays for vector
control, together with the three recommended practices for HLB management. Those sources of inoculum are commonly confused with commercial citrus trees maintained treated by insecticides in neighbor areas. However, real attention and efforts have to be used to localize and remove these rutaceous plants, never sprayed for vector control, located inside and outside of commercial properties and until now underestimated as important sources of HLB inoculum.
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